Exploring Lignocellulosic Biomass!

Challenges and Opportunity for Bioeconomy

26 - 29 June 2018 – Reims, France

Foreword

Dear ELB 2018 participants,

It is a great honour to welcome you in Reims at the ELB 2018 meeting. As some of you remember, the first ELB meeting was organized in Reims in 2016 to celebrate the 15th anniversary of the FARE laboratory. It was a big challenge for us to shape this meeting which was considered as successful due to the presence of renowned speakers and numerous attendees.

During the last two years, the paradigms of global bioeconomy and circular economy have matured in many countries, emphasizing the need to tune efficiently and hybridize the use of fossil and renewable carbon resources to fight climate changes and to create new economical assets. This has reinforced the importance of organizing scientific meetings dedicated to biomass transformation, and in particular of lignocellulose, which is the world ubiquitous resource for energy, materials, polymers and molecules for the future.

That is why ELB 2018 has integrated a session on bioeconomy to open the meeting, focusing on the challenges that our societies face today. The following sessions will be focused on new advances in understanding the complexity of lignocellulose (Session 2), its transformation in biotechnological processes (Session 3) and in soils (Session 4), and finally valorisation as bioproducts (Session 5) and materials (Session 6).

With nearly 40 oral communications and more than 130 attendees, the environment is perfectly appropriate to debate fascinating interdisciplinary scientific questions around lignocellulose and its importance for the future of agriculture, food, health and global wellness.

Finally we would like to acknowledge the support of our partners and sponsors that has allowed us to organize this international conference and to invite several world-recognized scientific leaders. Let's explore the lignocellulosic biomass together now!

I hope you will all have fruitful discussions and enjoy your stay in Reims.

Have a nice meeting!

Gabriel Paës, Chairman of ELB 2018

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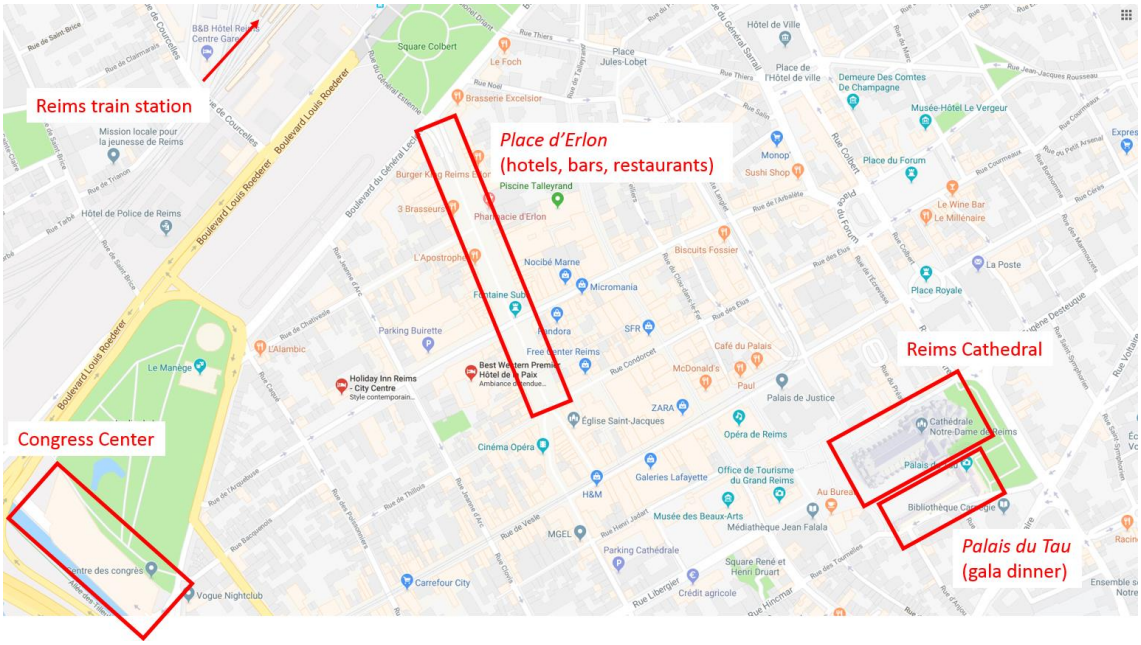
Practical information

Meeting venue

The Congress Center ("Centre des Congrès") is located 12 boulevard du Général Leclerc, 51100 Reims. Hotels in the "Place d'Erlon" or around are 5 minutes walk and also very close to the Reims train station. Numerous car parkings are available.

Reims Cathedral and Palais du Tau

Both monuments are 10 minutes walk from the Congress Center.



Programme

Tuesday 26 June 2018

12:00 Opening of the welcome meeting desk

14:00 Opening remarks by Gabriel PAËS, Chairman of ELB 2018

Forewords by:

Monique AXELOS, INRA Scientific Director for Food, Nutrition & Bioeconomy

Laurent LUCAS, University of Reims Champagne-Ardenne Vice-President in charge of Research

14:40 Session 1 – Biorefining for Bioeconomy

Chairperson: Bernard KUREK. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France

14:40 Keynote lecture: **Anne-Christine RITSCHKOFF**. VTT - Espoo, Finland
Biobased innovations drive circular bioeconomy - a Finnish perspective

15:25 Keynote speaker: **Antoine MISSEMER**. CIRED, CNRS - Paris, France
Energy, environment and natural capital: an economic and historical perspective

16:10 *Coffee break*

16:40 **Nicolas BEFORT**. Chair in Industrial Bioeconomy, NEOMA Business School, European Center in Biotechnology and Bioeconomy - Reims, France
The bioeconomy, biotechnology, and the transition to sustainability

17:00 **Manuel MORALES**. Chair in Industrial Bioeconomy, NEOMA Business School, European Center in Biotechnology and Bioeconomy - Reims, France
Industrial Symbiosis, a dynamic transitional strategy to a Bio-based Economy

17:20 **Jacky VANDEPUTTE**. French Bioeconomy Cluster (IAR) - Laon, France
What has been accomplished in the bioeconomy in France?

17:40 **Julie WOHLFAHRT**. ASTER, INRA - Mirecourt, France
Bioeconomy systems sustainability assessment: embracing complexity

18:00 *End of Session 1*

End of the day

Wednesday 27 June 2018

09:00 Session 2 – Structural and chemical complexity of lignocellulose

Chairpersons:
Estelle BONNIN. BIA, INRA - Nantes, France
Brigitte CHABBERT. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France

09:00 Invited speaker: **Peter CIESIELSKI**. NREL - Golden, USA
Integrating advanced microscopy, structural modelling, and multiphysics simulation to understand transport phenomena and conversion processes in lignocellulosic biomass

09:45 **Fabienne GUILLON**. BIA, INRA - Nantes, France
Multispectral autofluorescence analysis, a promising approach to track tissue origins of particles in ground grass lignocellulosic biomass

10:05 **Julia PARLATORE LANCHA**. LGPM, CentraleSupélec - Pomacle, France
Parietal modifications of lignocellulosic biomass subjected to hydrothermal pretreatment observed by Raman micro-spectrometry

10:25 *Coffee break*

10:55 **Simon HAWKINS**. UGSF, University of Lille/CNRS - Lille, France
A chemical reporter strategy for studying lignification in plants

11:15 *Sponsor presentation: Interchim by **Julien LEFEBVRE***

11:25 **Alain BOURMAUD**. IRDL, Université Européenne de Bretagne - Lorient, France
Plant fibre cell walls characterization by peak-force quantitative nano mechanics technology

11:45 **Carlos MARCUELLO**. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France
Interfacial forces between lignocellulosic polymers by Single Molecule Force Spectroscopy: Impact of the coverage AFM- tip

12:30 *Lunch*

14:00 ~~Keynote speaker: **Art RAGAUSKAS**. Department of Chemical & Biomolecular Engineering, University of Tennessee - Knoxville, USA~~
~~*Fundamentals of biorefining: Today and Tomorrow* (video conference)~~

14:45 *End of Session 2*

14:45 Session 3 – Physical, chemical and biological deconstruction of lignocellulose (to be continued on Thursday 28 June)

Chairpersons:
Fabienne GUILLON. BIA, INRA - Nantes, France
Caroline RÉMOND. FARE, University of Reims Champagne-Ardenne/INRA - Reims, France

14:45 Keynote speaker: **Anne S. MEYER**. Technical University of Denmark, Denmark
Assessing laccase catalyzed lignin modification: EPR measurements and mediator effects

15:30 **Jean-Guy BERRIN**. BBF, INRA/Aix-Marseille Université - Marseille, France
Lytic oxidative enzymes from fungal biodiversity as innovative tools for plant (hemi)cellulose processing

15:50 **Harivony RAKOTOARIVONINA**. FARE, University of Reims Champagne-Ardenne/INRA - Reims, France
Genomic and transcriptomic analyses of a hemicellulolytic thermostable bacterium reveal its potentiality and adaptability for an efficient biomass fractionation

16:10 *Coffee break*

16:40 **Estelle BONNIN**. BIA, INRA - Nantes, France
Synchrotron time-lapse imaging of lignocellulosic biomass hydrolysis: tracking enzyme by autofluorescence, and cell walls modifications by infrared microspectroscopy

17:00 *Sponsor presentation: Realcat by **Egon HEUSON***

17:10 **Nicolas BROSSE**. LERMAB, Université de Lorraine/INRA - Nancy, France
The steam explosion process for lignocellulosics pretreatment: beyond bioethanol

17:30 **Claire MAYER**. IATE, INRA/CIRAD/University of Montpellier - Montpellier, France
Preserving the structural variability in maize stalk through dry fractionation processes

17:50 **Ezinne ACHINIVU**. Chaire ABI, AgroParisTech - Pomacle, France
Extraction and recovery of sinapic acid from oleaginous biomass (mustard bran): a sustainable access to a valuable phenolic platform molecule

18:10 *End of Session 3 to be continued on Thursday 28 June*

End of the day

Thursday 28 June 2018

8:45 Session 4 – Lignocellulose as a source of organic matters in soils

Chairpersons:
Gwenaëlle LASHERMES. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France
Sylvie RECOUS. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France

8:45 Keynote speaker: **Claire CHENU**. ECOSYS, AgroParisTech/INRA - Thiverval-Grignon, France
Closing the loop between soils and lignocelluloses to address global challenges

9:30 Invited speaker: **Petr BALDRIAN**, Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Republic Czech Academy of Sciences. Prague, Czech Republic
Lignocellulose in forest soils and its microbial decomposers

10:05 **Fida MRAD**. AGHYLE, Polytechnic Institute UniLaSalle - Rouen, France
Soil microbial response to sunflower residue and corresponding agromaterial inputs

10:25 **Laurent BLEUZE**. FARE, INRA/University of Reims Champagne-Ardenne - Reims, France
Microbial colonization of hemp mulches during field retting at the soil surface

End of Session 4

10:45 *Coffee break*

11:15 Session 3 – Physical, chemical and biological deconstruction of lignocellulose

Chairpersons:
Fabienne GUILLON. BIA, INRA - Nantes, France
Caroline RÉMOND. FARE, University of Reims Champagne-Ardenne/INRA - Reims, France

11:15 Invited speaker: **Thomas FARMER.** University of York - York, UK
Lignocellulose deconstruction and the importance this has on maximising the use of chemical functionality in bio-based platform molecules

11:50 **Eric HUSSON**. GEC, CNRS/University of Picardie Jules Verne - Amiens, France
Biorefinery strategies based on room temperature ionic liquids, hydrolases and their synergism with other pretreatments

12:10 **Egon HEUSON**. University of Lille - Lille, France
Innovative high-throughput microplate approach for cell wall degrading enzyme production based on fungi and lignocellulosic raw biomass interaction

12:30 *End of Session 3*

12:30 *Lunch*

14:00 *Selected Flash Talks of posters (3 min each)*

Poster 2 by **Virginie STEINMETZ**
Analytical characterization of a soluble lignin fraction from thermomechanical softwood treatment: toward a biorefinery concept

Poster 3 by **Aya ZOGHLAMI**
Spatio-temporal imaging of lignocellulosic biomass deconstruction and correlation with the enzymatic hydrolysis

Poster 8 by **Alexandre CLERCQ**
Performance of a stoichiokinetic model to predict the degradation of lignocellulose by reactive oxygen species and the formation of valuable compounds

Poster 12 by **Quentin CZERWIEC**
Ligninolytic potential of Thermobacillus xylanilyticus *for the production of aromatic molecules*

Poster 14 by **Hasna NAIT M'BAREK**
Lignin decomposition for enhanced bioethanol production: Improving the process using a bio-pre-treatment with the fungus isolate Humicola grisea *from central Morocco*

Poster 18 by **Varunesh CHANDRA**
Gaseous emissions of nitrogen species from decomposing crop residues: construction and calibration of a novel model

Poster 26 by **Clémentine VEROVE**
New sugar-based amphiphilic compounds with surfactant or gelling properties – focus on xylose from ligno-cellulosic biomass

Poster 30 by **Feng CHEN**
Thermodynamics and kinetics screening of cellulose fiber dissolution in ionic liquids

14:30 *Poster session*

17:00 *End of the poster session*
18:00 *Optional upon registration: visit of the Reims Cathedral*

19:00 *Optional upon registration: Gala dinner at the Palais du Tau*

Friday 29 June 2018

9:00 Session 5 – Lignocellulose as a source of biomolecules for energy and platforms molecules

Chairpersons:
Xavier COQUERET. ICMR, CNRS/University of Reims Champagne Ardenne - Reims, France
Gabriel PAËS. FARE, INRA/ University of Reims Champagne Ardenne - Reims, France

9:00 Keynote speaker: **Ed de JONG.** Avantium - Amsterdam, The Netherlands
Zambezi Biorefinery: "Pure" glucose from 2nd generation feedstocks

9:45 Invited speaker: **Warwick RAVERTY**. CIRCA - Melbourne, Australia
Levoglucosenone: à la recherche de chimie perdue

10:20 **Xiao ZHANG**. Washington State University - Richland, USA
New approach toward lignin conversion to fuel and chemicals

10:40 *Coffee break*

11:10 **Kati GÖRSCH**. Deutsches Biomasseforschungszentrum gemeinnützige GmbH - Leipzig, Germany
Hydrothermal conversion of lignocellulosic sugars to furans

11:30 *Sponsor presentation: Futurol by **Frédéric MARTEL***

11:40 **Caroline RÉMOND**. Chaire AFERE, FARE, University of Reims Champagne-Ardenne/INRA – Reims, France
Enzymatically-synthesized alkyl pentosides and pentose-based esters, as surfactants of interest

12:00 **Florent ALLAIS**. Chaire ABI, AgroParisTech - Pomacle, France
From sawdust to high value-added fine chemicals: a France-Australia-USA success story

12:20 *End of Session 5*

12:30 *Lunch*

14:00 Session 6 – Biobased materials from lignocellulose

Chairpersons:
Alain BOURMAUD. IRDL, Université Européenne de Bretagne - Lorient, France
Simon HAWKINS. UGSF, University of Lille - Lille, France

14:00 Keynote speaker: **Richard GROSS**. Rensselaer Polytechnic Institute, USA
High performance biobased structural epoxy resins derived from levulinic acid (video conference)

14:45 Invited speaker: **Armand LANGLOIS**. ENERLAB, Canada
Iso-Lignin® technology, lignin base polyurethane: challenges and opportunities

15:20 **Elise GERBIN**. FARE, University of Reims Champagne-Ardenne/INRA – Reims, France
Lignin cross-linking in cellulose nanocrystal based films by Fenton reaction: mechanism and properties

15:40 **Amandine VIRETTO**. IATE, INRA/CIRAD/University of Montpellier - Montpellier, France
Valorization of lignocellulosic fibers derived from park and garden wastes for biocomposites applications

16:00 *Coffee break*

16:30 **Clovis BERTHELIN**. IMT, Polymers and Composites Technology & Mechanical Engineering Department - Douai, France
Water-assisted extrusion compounding process to reduce the total volatile organic compounds in natural fibre-filled composites for automotive interior applications

16:50 **Julie BOSSU**. Institute of Paper, Pulp and Fibre Technology, Graz University of Technology - Graz, Austria
GuyaValoFibres: exploring the potential of Amazonian unvalorized ligno-cellulosic resources for fibres-based composites applications

17:10 **Arnaud DAY**. Fibres Recherche Développement - Troyes, France
Quality management of hemp straw used in agro-materials

17:30 *End of Session 6*

17:30 *Closing remarks*

17:45 *End of ELB 2018 meeting*

Exploring lignocellulosic biomass!
Challenges and opportunities for bioeconomy
26 - 29 June 2018 in Reims - France

Lecture Abstracts

Session 1
Biorefining for Bioeconomy

Session 2
Structural and chemical complexity of lignocellulose

Session 3
Physical, chemical and biological deconstruction of lignocellulose

Session 4
Lignocellulose as a source of organic matters in soils

Session 5
Lignocellulose as a source of biomolecules for energy and platforms molecules

Session 6
Biobased materials from lignocellulose

Keynote lecture

Biobased innovations drive circular bioeconomy - a Finnish perspective

Anne-Christine Ritschkoff

VTT Technical Research Centre of Finland Ltd, Espoo, Finland

Finland is a country of forest, around 78% of the total area is covered by forest. The annual growth of forest exceeds 100 Mm^3, from which 60% is in use. Sustainable forest-based Bioeconomy has a key role in Finland´s economy and wellfare. The expected output of Bioeconomy is 100 billion € by 2025. The Finnish Bioeconomy strategy targets to pave the way for competitive environment, establishment of new businesses, establishment of strong knowledge basis and sustainable use of raw materials [1]. Innovation and collaboration along the innovation value chain have a crucial role in maximizing the output for optimal use of biomass and sustainable Bioeconomy. New value added forest-based products, such as bio composites, alternatives to fossil plastics, resins & chemicals, building blocks and fibre products, can double the value of forest sector by 2030 in Finland. Circular bioeconomy have great positive impact on climate change and challenges connected to the increasing need of materials, food & feed, energy and services.

The inherited properties of cellulose - bio-based, biodegradable, recyclable - makes it a future super material. Cellulose structure enables excellent product performance, ability to modify and construct new materials and novel material design. Currently, nano- and micro cellulose, cellulose films for packages, 3D-printed products and cellulose textile fibres are on the verge of a breakthrough.

[1] Sustainable growth from bioeconomy, The Finnish Bioeconomy Strategy, May 2014

Keynote lecture

Energy, environment and natural capital: an economic and historical perspective

Antoine Missemer

CNRS, CIRED, Paris, France

This presentation, based on a recently published book [1], will explore how economists historically incorporated energy issues in their tools, concepts and theories, with a particular focus on fossil fuels. The economic conception of fossil fuels changed overtime, from a systemic perspective to a more reduced, sector-oriented or even individual perspective. Energy dependency, competitiveness and economic development, which were major concerns in the middle of the 19th century, left the place to theoretical questions on the optimal allocation of ore resources for one producer, on the road to synthetic and formalized models elaborated in the 1930s. That movement was not linear. It occurred through various forward and backward steps, with theoretical tracks sometimes abandoned. The reasons for these evolutions are to be found both in economic and environmental history (sociotechnical change, decrease of the perceived biophysical constraints) and in the history of economic thought and analysis (development of marginal utility, of capital theory, use of mathematical tools). This presentation will define the role of each of these factors in the history of energy economics, and will give some insights to understand why economists (still today) deal with environmental and energy issues in such a peculiar way in comparison with other scientists.

[1] Missemer A. *Les Économistes et la fin des énergies fossiles (1865-1931)* [*Economists and the End of Fossil Fuels (1865-1931)*], 2017, Paris: Classiques Garnier, 225 pages.

Session 1: Biorefining for Bioeconomy

Oral communication

The bioeconomy, biotechnology, and the transition to sustainability

Nicolas Befort[1], Martino Nieddu[2]

[1]Chair in Industrial Bioeconomy, Neoma Business School, Reims, France

[2]REGARDS (EA 6292), University of Reims Champagne Ardenne, Reims, France

Since 2012, the term 'bioeconomy' has increasingly appeared in the literature (Bugge et al., 2016), and in public policy, with the publication of technological roadmaps for countries, regions, industries and value-chains (McKormick and Kautto, 2013). Despite the importance of expectations that the concept will help achieve the transition to sustainability, the meaning of the bioeconomy is not clear. The OECD defines the bioeconomy as an economy driven by biotechnologies, involving the use of genes and complex cell processes, for a variety of sectors (industry, health, agriculture, etc.), and producing a third Industrial Revolution. Meanwhile, the European Commission (EC) characterise the bioeconomy merely as processes transforming renewable resources from agriculture, forestry, fisheries, food, pulp and paper production, and parts of the chemistry, biotechnology and energy sectors. Among these processes, the common denominator linking industries is not biotech but the concept and role of the 'biorefinery', which is a central artefact transforming the biomass using different technologies drawn together in complex knowledge bases (van Lancker et al., 2016). Hence, at least, two visions of the bioeconomy coexist.

The aim of this research is to unravel the links between biotechnology and the bioeconomy. While the field of biotechnology has been well known since Kenney (1986), and Arora and Gambardella (1990), the literature lacks a clear characterization of 'the bioeconomy' in terms of innovative economic activities. To fill this gap and compare biotechnology and the bioeconomy, we consider these two fields as sociotechnical regimes (Geels, 2002). In the first part of the study, we give an historical and institutional account of the differences between the sociotechnical regimes of the biotechnology and the bioeconomy. We conclude that the sociotechnical regime of biotechnology is mainly technology-driven, whereas the bioeconomy is mission-driven toward a 'great transition' toward the use of renewable resources.

In the second part of the study, we highlight the internal coherence of the two regimes through three historical qualitative case studies of emblematic bioeconomy products. From this analysis, we draw three conclusions and research avenues. First, there is an emerging sociotechnical regime of the bioeconomy. It opens the way to further research to identify its specificities and organizational modes since it covers food and materials production. Second, this regime may be plural and under tension because of competing logics. So there is an issue in identifying these logics. Third, bioeconomy public policies mainly focus on production side. There is an issue in designing and implementing consumption side policies.

Bugge, M., Hansen, T., Klitkou, A., 2016. What Is the Bioeconomy? A Review of the Literature. Sustainability 8, 691. https://doi.org/10.3390/su8070691

Fevolden, A., Coenen, L., Hansen, T., Klitkou, A., 2017. The Role of Trials and Demonstration Projects in the Development of a Sustainable Bioeconomy. Sustainability 9, 419. https://doi.org/10.3390/su9030419

Geels, F.W., 2002. Technological transitions as evolutionary reconfiguration processes: a multi-level perspective and a case study. Research Policy 31, 1257–1274

McCormick, K., Kautto, N., 2013. The Bioeconomy in Europe: An Overview. Sustainability 5, 2589–2608

Van Lancker, J., Wauters, E., Van Huylenbroeck, G., 2016. Managing innovation in the bioeconomy: An open innovation perspective. Biomass and Bioenergy 90, 60–69

Session 1: Biorefining for Bioeconomy

Oral communication

Industrial Symbiosis, a dynamic transitional strategy to a Bio-based Economy

Therese Bennich[1, 3], Manuel E. Morales[2,3]

[1]University of Stockholm, Department of Physical Geography, SE-106 91, Stockholm, Sweden

[2]NEOMA Business School, Chair in Industrial Bioeconomy, 59 rue Pierre Taittinger, 51100, Reims, France

[3]Clermont Auvergne University, Research Center on International Development Studies (CERDI), 65 Boulevard François Mitterrand, 63000 Clermont-Ferrand, France

The normative question "What are the causal structural patterns that drives the transition to a bio-based economy?" has received attention of researchers. This study provides an overview of strategies that could facilitate the transition to a Bio-based economy through the implementation of Industrial Symbiosis (IS) process, using system dynamics to integrate an environmental, social and economic analysis to assess future and current opportunities to achieve sustainability and test the system's vulnerable points. Based on the regional analysis of the lignocellulose cluster development in Norrköping, located in the province of Östergötland, in the south-eastern part of Sweden, the results identify the key strategic actors and found empirical evidence of a transition, validated by the causal relationship of energy behaviour patterns and the structural transition to a Bio-based economy, presented in the form of a System Dynamic model. We consider that the IS project at Norrköping has been a dynamic platform of scenario modelling and policy tests, exploring potential future patterns, risk factors, and opportunities to further expand the sustainable bio-based economy in the region. Concluding that the dynamic transition strategy to a bio-based economy has been fully addressed by the following successful mechanisms in Norrköping: [1] the construction of new partnerships in the forest industry between private and public, [2] the willingness of the stakeholders to encompass a compelling interactive learning process and [3] the innovation shared structure that decrease transaction costs and increase flexibility.

[1] Aid, G., et al., 2015. Looplocal – a heuristic visualization tool to support the strategic facilitation of industrial symbiosis. J. Clean. Prod., Special Volume: Support your future today! Turn environmental challenges into opportunities. 98, 328–335. https://doi.org/10.1016/j.jclepro.2014.08.012

[2] EU, 2012. Innovating for Sustainable Growth: A Bioeconomy for Europe [WWW Document]. URL http://ec.europa.eu/research/bioeconomy/pdf/official-strategy_en.pdf (accessed 1.13.17).

[3] Formas, 2012. Swedish research and innovation strategy for a bio-based economy. Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, Formas, Stockholm.

[4] Hatefipouret al., 2011. The Händelö area in Norrköping; Sweden. Does it fit for Industrial Symbiosis development?, in: World Renewable Energy Congress-Sweden; 8-13 May; 2011; Linköping; Sweden. Linköping University Electronic Press, pp. 3468–3475.

[5] Norrköpings kommun, 2017. Norrköping i siffror [WWW Document]. URL https://www.norrkoping.se/download/18.3ef6b1d158f1bd46e11c377/1488791488534/NKPG_isiffror2016_sve.pdf (accessed 3.2.17).

Session 1: Biorefining for Bioeconomy

Oral communication

What has been accomplished in the bioeconomy in France?

Jacky Vandeputte

French Bioeconomy Cluster (IAR)

As the French Bioeconomy Cluster, IAR and its members have been strongly contributing to the development of the national Bioeconomy Strategy and the action plan which has been published recently. It aims to propose a coherent framework for all initiatives undertaken in the sector, supporting an increased and sustainable mobilization of locally produced biomass to boost its efficient use and valorization to answer our needs (food and non-food). The French bioeconomy is very much linked to local territories and the valorization of locally produced biomass in integrated biorefineries. French industries based around the living world are key actors in the bioeconomy. They are already engaged in innovative approaches, helping new uses to emerge. The different sectors of agriculture are engaged in the production of renewable energy. The forestry & wood sector proposes a variety of uses for biomass, ranging from construction timber to energy, and including industrial lumber and innovative molecules. New materials and molecules are produced from agricultural and forestry biomass… All actors have been able to benefit from support for innovation. The French program of investment for the future has led since 2010 to allocation of €250m in support of Bioeconomy projects. The resulting strategy is aimed at strengthening all value chains at the same time.
Some practical examples of the application of Bioeconomy principles and open innovation R&D&I project will be described, old and new value chains and drivers will be tackle. New resource of Protein, algae refineries, insect's fractionation, plant protection….

[1] A bioeconomy strategy for France

Oral communication

Bioeconomy systems sustainability assessment: embracing complexity

Julie Wohlfahrt[1], Fabien Ferchaud[2], Benoît Gabrielle[2], Caroline Godard[4], Bernard Kurek[5], Chantal Loyce[6], Hélène Preudhomme[4], Olivier Therond[7]

[1]SAD-ASTER, INRA, Mirecourt, France

[2]Agro-Impact, INRA, Laon, France

[3]UMR EcoSys, INRA-AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France

[4]Agro-Transfert Ressources et Territoires, Estrees-Mons, France

[5]FARE Laboratory, INRA, University of Reims Champagne-Ardenne, Reims, France

[6]UMR Agronomie, INRA-AgroParisTech, Thiverval-Grignon, France

[7]UMR LAE, INRA-Université de Lorraine, Colmar, France

The European Union sets the objective of a sustainable bioeconomy as "to ensure an adequate supply of food and products of biological origin that are safe, healthy and of high quality by developing sustainable and resource-efficient production systems, related ecosystem services, restoration of biological diversity, competitive and economical processing and marketing chains in carbon". Bioeconomy appears as a key policy to integrate the great societal issues of climate change, food security, fossil fuel dependence, scarcity of natural resources and territorial and industrial development on a number of scales, from small territory to national scale, as sustainable production and use of biomass are inherent to the bioeconomy proposition. One major challenge for bioeconomy is then to be able to assess how its development will successfully achieve these different objectives in concrete situations, from biomass production to recycling processes. Several authors highlight the lack of knowledge regarding the reality of bioeconomy systems that limits the development of sustainable bioeconomy projects in territories.

Bioeconomy as a system is a relatively new concept that encompasses several sectors and disciplines (e.g.: agriculture, forestry, industry). The scientific community is then still quite scattered from a process to a resource approach. The same conclusions can be established regarding stakeholders that are mostly focused on one component of the bioeconomy system (e.g.: forest-wood chains, biofuels production). Bioeconomy systems are also characterized by a high flexibility of resources, processes, stakeholders or transformation chains. Understanding the whole bioeconomy system and proposing sustainable organization to optimize biomass productions and uses requires accounting for the high level of complexity inherent to bioeconomy systems.

There is a need for a specific framework that deciphers bioeconomy systems in order to help their integrated assessment. Starting with territorial bioeconomy systems, we propose a description to help the concrete sustainability assessment and highlight the potential locks and levers for bioeconomy development.

Keynote lecture

Fundamentals of Biorefining: Today and Tomorrow

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Although today's low-energy costs have reduced the commercialization of cellulosic ethanol production, we have seen the development of commercial cellulosic-ethanol plants on a global basis employing either the thermochemical or the biological technology platform. Despite these successes, technical challenges remain which hinder broad acceptance of biofuels which for the biological platform include the recalcitrance of biomass and what to do with lignin. Our research studies and others have clearly shown that the recalcitrance of biomass is a multi-tiered effect due to the complex nature of the plant cell wall which can include: Biomass particle size and porosity, Cellulose, Hemicellulose and Lignin. Our on-going studies have shown that the natural variance of the plant-cell wall can influence recalcitrance and chemical pretreatments substantially altering the structure of the cell-wall components further reducing recalcitrance. Analysis of cellulose, hemicellulose and lignin from low and high recalcitrance biomass feedstocks including switchgrass and poplar, before and after chemical pretreatment, is one of the most promising methodologies to investigate and dissect the fundamental mechanisms of recalcitrance. Employing these protocols, we have shown that acidic and neutral pretreatments usually provide a biomass resource with increased crystallinity which is less reactive to cellulose and thereby not a beneficial component to reducing recalcitrance. The loss of hemicelluloses and changes in structure of lignin, during these pretreatments, certainly provides a more reactive biomass for biological deconstruction. Depending on the severity of the pretreatment, lignin undergoes a series of competing depolymerization reactions cleaving B-O-aryl ethers which can then undergo further condensation reactions. This presentation will examine how advanced NMR and GPC techniques can be used to investigate the changes in bulk cell wall chemistry and how ToF-SIMS can be used to monitor changes on the surface of biomass before and after pretreatment [1-5].

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Invited lecture

Integrating advanced microscopy, structural modelling, and multiphysics simulation to understand transport phenomena and conversion processes in lignocellulosic biomass

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Biomass conversion processes require coordination of complex physiochemical processes that occur over multiple length and time scales. Biochemical deconstruction relies on the penetration of catalysts, including enzymes and other small molecules, into the cell wall. Thermochemical conversion processes, such as pyrolysis and gasification, require rapid heat transfer to drive high-temperature depolymerization reactions. Optimization of these processes requires a detailed understanding of how the transport of heat and mass is affected by the complex, hierarchical structure of lignocellulosic biomass. Developing integrated multiscale simulations for these processes will allow for high-throughput investigations of various conversion scenarios in silico and provide some predictive utility regarding the efficacy of new processing paradigms. In this presentation, I will describe our efforts toward multiscale simulations of transport phenomena and biomass conversion in the context of structurally and compositionally detailed model geometries. First, I will present recent progress with coupled molecular and mesoscale simulations that incorporate biopolymer structure at the cell wall scale. Molecular dynamic simulations are used to evaluate diffusion coefficients of small molecules through cell wall biopolymer assemblies. These are coupled to mesoscale models that account for the complex organization of cellulose fibrils, lignin, and hemicellulose at the cell wall scale. Second, I will describe particle-scale models that incorporate higher-order geometric features, such as cellular geometry and anisotropic particle shapes, in the context of thermochemical conversion simulations. All of the structural models employed in these simulations are informed by multimodal analytical microscopy which will be described throughout the presentation.

Oral communication

Multispectral autofluorescence analysis, a promising approach to track tissue origins of particles in ground grass lignocellulosic biomass

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In a biorefinery plant diagram, a grinding step is usually the prerequisite for biological conversion, allowing material densification and homogenization for transport and storage, or an increase of the accessible surface. Powders produced from plant biomass are heterogeneous in relation to native plant heterogeneity, and during grinding, dissociation often occurred at the tissue scale. The distribution of tissue within a single particle is required to understand, control and optimize the plant deconstruction through dry fractionation process. Therefore the tissular origin of powders must be identified at the particle level. If recognition of tissues is easy in sections of plant organs, it is not straightforward in powders. Taking advantage of the autofluorescence properties of cell wall components, we propose to use multispectral image analysis to identify the tissular origin of lignocellulosic particles. Maize stem was chosen as model of grass lignocellulose. The fluorescence variability of maize stem sections was first investigated. Images of the stem sections were acquired and chemometric approaches was implemented to reveal fluorescence variability without any *a priori*. Fluorescent profiles were extracted for the main tissues. Images of ground tissues, isolated from the same stem, were acquired using the same procedure. The fluorescence intensity profiles were analysed using principal component analysis. Similar variability was found in fluorescence profiles extracted from powders and tissues confirming the potential of fluorescence multispectral imaging to predict the tissue origin of particles.

Oral communication

Parietal modifications of lignocellulosic biomass subjected to hydrothermal pretreatment observed by Raman micro-spectrometry

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In order to overcome the recalcitrant macromolecular structure of lignocellulosic biomass and to make carbohydrates sufficiently accessible for enzymatic hydrolysis, a pretreatment is required. This pretreatment can consist of a hydrothermal treatment, followed or not by steam explosion[1]. In this work, changes in ultrastructural characteristics of poplar's cell wall caused by hydrothermal treatment were studied. To ensure the homogeneity of the treatment throughout the sample, poplar cubes with a characteristic size of less than one centimeter were treated using an in-house developed device[2]. Cell wall changes have been assessed by a microscopic study of its components before and after the process. Raman micro-spectrometry is known to be a powerful tool for chemical-mapping[3]. However, in comparison with native wood, the sample preparation of biomass after pretreatment is challenging due to the weak cohesion of cells and cell wall layers. To overcome this issue, we developed a new sample preparation method, suitable for both kinds of sample. Two-dimensional chemical maps were obtained by integration over wavelength ranges with strong Raman signal using an Alpha 300R+ confocal Raman microscope from Witec. Lignin was detected by the peak around 1600 cm^{-1} which corresponds to the aromatic C=C bond[3]. For untreated wood, as expected, the areas with higher lignin concentration are in the cell corner and middle lamella, which is particularly visible at triple points. Integration around 1120 cm^{-1} corresponds to C-O-C symmetric linkage found in carbohydrates[3] and enables the identification of the so-called holocellulose, which includes cellulose and hemicellulose. It is present in higher concentration in the S2 layer of the cell wall. The comparison with treated biomass reveals the deformation of cell wall and sub-parietal chemical changes. In order to evaluate the kinetics of these chemical changes, this method is applied to samples obtained after different treatment durations.

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Oral communication

A chemical reporter strategy for studying lignification in plants

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The quality of lignocellulosic biomass for a wide range of different industrial uses (biofuels, composite materials, textiles, paper etc.) is related to the chemical composition of the plant cell wall. Cell walls are dynamic structures that are progressively constructed and modified during plant growth and a better understanding of the factors affecting final composition of biomass at harvest can only be achieved through more knowledge about the dynamics of cell wall polymer formation. Obtaining this knowledge *in vivo* is usually hampered because of a lack of suitable probes. Although fluorescence-tagged monolignols have been developed for lignin [1], these often present the disadvantage of the tag being larger than the tagged molecule thereby potentially interfering with the lignification process. More recently, coniferyl alcohol monolignols tagged with clickable azide or alkyne tags have been used in a bioorthogonal chemical reporter strategy on the model species Arabidopsis [2], [3]. In this strategy, an analogue of the biomolecule of interest is modified with a biocompatible chemical function and is metabolically incorporated into the target biomacromolecule where it functions as a 'chemical reporter'. The incorporated chemical reporter is then visualized by fluorophore tagging initiated by a bioorthogonal chemistry reaction. We have recently [4], [5] developed an original Bioorthogonal Labeling Imaging Sequential Strategy (**BLISS**) to visualize and analyze the incorporation of both *p*-hydroxyphenyl (H) and guaiacyl (G) units into lignin *in vivo* with a combination of strain-promoted and copper-catalyzed azide-alkyne cycloadditions (**SPAAC**/**CuAAC**). We are currently using the BLISS strategy to study lignification dynamics in the flax fiber crop and other plants by confocal fluorescence microscopy.

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Oral communication

Plant fibre cell walls characterization by Peak-Force Quantitative Nano Mechanics technology

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The industrial interest for plant bast fibres used as composite reinforcements increases. The tensile properties of the elementary fibres are well described in the literature [1], but additional information at the cell wall scale is needed to better understand the relationship between their ultrastructure and their mechanical performance.

Indeed, an elementary flax fibre is itself assumed to be a composite because of its specific ultrastructure made up of several cell walls and layers. The S2 layer of the secondary cell wall, which mainly controls the longitudinal mechanical properties, is composed of ≈70% oriented cellulose microfibrils embedded in an amorphous polysaccharide matrix containing hemicelluloses and pectins [2]. During plant development, the secondary cell wall of the outer bast fibre bundles firstly becomes thickened, while complete filling takes around two months. Consequently, during fibre development, the degree of maturity may differ between the outer and inner fibres. Fibres can thus have a skin-core like ultrastructure, e.g., the so- called G and Gn sub-layers in the developing secondary cell wall [2].

The aim of this work is to present results from manipulations on plant cell walls performed in atomic force microscopy (AFM) associated with PeakForce Quantitative Nanomecanic (PF-QNM) mode. Mechanical mapping within the growing flax cell walls are showed as well as on mature fibre after thermal treatment to investigate the effect of a process cycle on flax fibres components. Finally, other examples of Peak Force QNM characterization on plant cell walls are shown, especially in palm or hemp fibres.

These studies validate the measurement method but also to suggest the full potential of the PF-QNM technology for the fine fibre characterization of plant cell walls. This tool provides an access to new information and open the way for many future applications.

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Session 2: Structural and chemical complexity of lignocellulose

Oral communication

Interfacial forces between lignocellulosic polymers by Single Molecule Force Spectroscopy: Impact of the coverage AFM- tip

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Nowadays, demands an increase of green products being environmental friendliness. In this context, lignocellulosic biomass provides a great opportunities for refineries to produce new cellulose based materials with large range of applications from automotive parts to 3D printer [1, 2]. In these materials, cellulose can be under the form of either pure cellulose nanofibril/nanocrystal or complex structure as lignocellulosic fiber containing a variety of polymers (cellulose, hemicelluloses, lignins,…) with distinctive physical and chemical characteristics. In this field, capitalizing on the interfacial forces between each lignocellulosic polymers in order to predict their cohesive behavior in (nano)material is required. For this reason, Atomic Force Microscopy (AFM) is presented as a convenient technique to achieve information on adhesion forces between components at nanoscale level. Chemical interactions between functionalized tip/tipless AFM levers and substrates can be interrogated by Single Molecule Force Spectroscopy (SMFS) mode. Strong skills in surface modification and polymer chemical conjugation are required for this purpose.
Building up to the previous expertise of our group [3], a broad range of different films (CNCs, hemicellulose as glucomannan or xylan and unmodified or laccase-oxidized lignin) has been deeply analyzed in terms of adhesion properties with functionalized tips with CNCs or synthetic lignin. Langmuir-Blodgett (LB) technique was used and compared with several chemical procedures to achieve this goal. Extensive analysis of how the coverage of the attached polymer on AFM-Tip influences on intermolecular interaction measurements with substrates previously detailed has been carried out. This unprecedented methodology in this field allows to precise control the measure of intermolecular interactions between lignocellulosic polymers in controlled environmental conditions (relative humidity, temperature). The promising results can open a gate to grasp the knowledge regarding the interactions of all these components and thus guide the conception of new materials with unique properties or enhance the lignocellulose biorefinery engineering.

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Keynote lecture

Assessing laccase catalyzed lignin modification: EPR measurements and mediator effects

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Laccases, EC 1.10.3.2, CAZY family AA1, are blue multicopper oxidases that catalyze oxidation of phenolic groups in lignin to phenoxyl radicals during reduction of O_2 to H_2O [1]. In fungal laccases, the T1 copper site in the enzyme and the redox potential define the ability of the enzyme to catalyze the abstraction of electrons from phenolic substrates, and the trinuclear cluster allows for the copper-catalyzed oxidoreduction reaction [2,3]. We used electron paramagnetic resonance spectroscopy (EPR) for real time measurement of enzymatic radical formation on different types of lignin by fungal laccases derived from *Trametes versicolor* and *Myceliophthora thermophila*, respectively, and showed that such measurements can be used to assay laccase catalyzed radical formation in lignin [4]. The radical formation activity was independent of the enzyme's redox potential [4]. By EPR on biorefinery lignin samples, we also examined the influence on laccase catalyzed radical formation of mediators, 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI), 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), presumed to act by shuttling electrons between the laccase and subunits in lignin that the laccase enzymes cannot approach directly. ABTS addition produced confounding EPR signals, but laccase-mediator treatments with 500 µM N-OH type mediators (HPI or HBT) did not affect the radical formation whereas high doses of 5 mM HPI or HBT surprisingly led to significantly *decreased* radical formation rates and lowered steady state radical concentrations [5]. Laccase-TEMPO treatment at a 5mM mediator dose significantly increased steady state radical concentration and rate of radical formation on beech organosolv lignin [5]. The data suggest that electron shuttling by mediators is not a significant general mechanism for enhancing laccase catalyzed oxidation of biorefinery lignin substrates. The EPR results thus provide both a new real time laccase assay and a new view on mediator functions in laccase catalyzed lignin modification.

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Oral communication

Lytic oxidative enzymes from fungal biodiversity as innovative tools for plant (hemi)cellulose processing

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Plant polysaccharides are the most abundant renewable carbon source on earth and are highly relevant to face tomorrow's environmental concerns. However, the technologies needed to convert plant carbohydrates into valuable products that can then be utilized in emerging biorefineries face several challenges due to the recalcitrance of biomass to enzymatic deconstruction. In this context, one of our objectives is to identify and characterize novel biocatalysts from fungal natural diversity. Fungal strains efficiently converting the polysaccharide fraction of biomass are deeply investigated using comparative post-genomic approaches [1-3]. Correlation between -omics data and markers of biomass recalcitrance enable us to select promising hydrolases and oxidases encountered in fungal secretomes. Special attention is given to lytic polysaccharide monooxygenases (LPMOs) that are considered as a breakthrough in the enzymatic degradation of biomass because they oxidatively cleave glycosidic linkages that render plant biomass more susceptible to hydrolysis by conventional cellulases [4-5]. The novel enzymes identified are heterologously expressed in *Pichia pastoris* taking advantage of the in-house medium-throughput expression system allowing small-scale expression of recombinant enzymes. The mechanism of action of the most efficient enzymes is studied in depth using multi-disciplinary approaches (i.e., biophysics, protein crystallography and bio-inorganic chemistry). These investigations give insights into the substrate specificity, catalysis and biological function of the targeted enzymes. Production of the most-promising enzymes is up-scaled in bioreactors to facilitate their integration into bioprocesses. Biotechnological applications are directed towards the improvement of plant biomass saccharification and the production of renewable materials.

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Oral communication

Genomic and transcriptomic analyses of a hemicellulolytic thermostable bacterium reveal its potentiality and adaptability for an efficient biomass fractionation

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Enzymatic reactions to transform biomass components require the identification of robust and efficient biocatalysts. Thermostable enzymes offer potential benefits in the hydrolysis of lignocellulose as they are more resistant to extreme bioprocessing. Hemicelluloses could form a physical barrier preventing the hydrolysis of cellulose polymers within lignocellulosic biomass. It is necessary to deconstruct them specifically to improve the biomass upgrading (by allowing accessibility of cellulases and by liberating components from hemicelluloses). *Thermobacillus xylanilyticus* is a Gram positive thermophilic and hemicellulolytic bacterium able to grow on several substrates including simple sugars (glucose, xylose…) and complex polysaccharides such as xylans [1]. This bacterium can grow onto a variety of plant derived feedstocks like wheat bran and straw by exhibiting rapid growth [2]. The hemicellulases produced by this bacterium are thermostable and able to release efficiently pentoses and ferulic acid from lignocellulosic biomass [3, 4]. We developed a combination of genomic, transcriptomic and other physiological (growth and enzyme production) approaches to determine the behavior of *T. xylanilyticus* while using lignocellulosic biomass as well as complex and soluble sugars. The size of the genome after sequencing was 4Mbp representing 3900 genes predicted. High lignocellulolytic potential was observed with 162 CAZYmes encoding (carbohydrate metabolism) and 114 oxido-reductases putative genes (that could play a role in the lignin biotransformation). *T. xylanilyticus* does not possess genes encoding for cellulase activities. A RNA-sequencing approach has been developed to study the expression of lignocellulolytic genes by the bacteria through its growth on two chemically-contrasted lignocellulosic biomasses (wheat bran and wheat straw) as well as on xylan and glucose The results demonstrate the involvement of seven identical core enzymes whatever the complex substrates to be deconstructed. The expression of other hemicellulolytic enzymes change with the substrates showing an adaptation of lignocellulolytic enzyme expression with the biomass used. These results indicate the importance of using different biomass sources to encourage the production of specific and tailor made enzymatic cocktails. The strategies identified by these combinatorial approaches could be applied for further efficient biotechnological deconstruction of lignocelluloses.

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Oral communication

Synchrotron time-lapse imaging of lignocellulosic biomass hydrolysis: tracking enzyme by autofluorescence, and cell walls modifications by infrared microspectroscopy

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The enzymatic conversion of lignocellulosic materials for the production of chemicals and fuels in replacement of petroleum feedstock is a very promising approach due to high enzyme selectivity and mild conditions of implementation. However, the efficiency of enzymatic degradation on plant cell walls is low. In the last decade, many efforts have been devoted to the understanding of biomass recalcitrance to enzymatic degradation but no consensus has been reached on the most important features explaining it. Biomass is most often considered as a bulk material and it would be helpful to take into account the diversity and heterogeneity of plant cell walls, and the dynamic of enzymatic degradation.

In the present work [1], the enzymatic degradation of lignocellulosic biomass has been imaged without labelling the enzyme or the cell walls thanks to synchrotron facilities. Excitation at 275 nm allowed autofluorescence recovery for protein and phenolics, and multichannel imaging to highlight concomitantly the presence of enzymes on cell walls and the changes of cell walls. Image analysis was used to quantify the variations of fluorescence intensity. In parallel, microfluidic FT-IR microspectroscopy allowed time-lapse tracking of local changes of the cell wall polysaccharides during degradation. The presentation will show the degradation dynamics by a cellulolytic/hemicellulolytic preparation. Consistent variations in the enzyme concentration were found locally in cell cavities and their surrounding cell walls. The quantification of fluorescence intensity showed that the enzymes were not evenly distributed and take into account the cell wall heterogeneity, not only from one cell type to another but also on a given cell.

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Oral communication

The steam explosion process for lignocellulosics pretreatment: beyond bioethanol

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Steam explosion (SE) is currently one of the most valuable and cost effective pretreatment technologies for cellulosic bioethanol production. After impregnation, biomass is treated with hot steam (around 200°C) under pressure (around 1.5-3 MPa) during few minutes followed by an explosive decompression. The effect of SE on biomass combines a chemical hydrolysis during the stream treatment and defibration during the explosive decompression to make the cellulose more amenable to enzymes. This technology is currently developped at the commercial scale (continuous processes) in Italy (beta renewables) and in the USA (Abengoa) for biofuel production from lignocellulosic feedstocks.

In this paper we will demonstate that beyong bioethanol, steam explosion could be considered as a versatile technology for lignocellulosics deconstruction for different advanced applications. The examples given include current on-going works from our group :

- the production of fine hemp fibers (cottonisation of hemp) for textile applications : the optimization of elementary water- or NaOH-impregnated hemp fibers extraction using SE is described. An original quantification method was developed in order to follow the defibration rate by image processing. Defibration was evaluated and optimized using systematical experimental method for the production of superior quality fibers with a low variability.
- the pretreatment by SE of phytoremediation lignocellulosic feedstocks (heavy metals contaminated) for the production of bioethanol and fibrous materials. The influence of the severity of the SE process on the composition in metals of the fibrous cellulosic residues was examined. The residual metal effect on the enzymatic hydrolysis of cellulose into glucose and on the fermentation step was also investigated.
- the extraction of biopolymers from biomass in control conditions. SE appeared to be a selective and non desctructive method for the extraction and solubilisation of high molecular mass biopolymers (hemicelluloses, proteins, lignin).

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Oral communication

Preserving the structural variability in maize stalk through dry fractionation processes

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The composition and the structure of biomass makes it a wonderful source of raw materials for the production of energy or bio based material. As examples, the most inner part (the pith) of corn stalk, an agricultural by-product, shows an alveolar structure, similar to those of expanded polystyrene, responsible of insulating properties [1,2]. On other example are the vascular bundles of the pith and the ring, which also insure, a mechanical support for the plant, making them particularly interesting for reinforcement of composite materials. One of the challenges is to recover each plant part without damaging its structure to preserve the original properties. This can be achieve by dry fractionation processes, which allow to dissociate plant structures at the relevant scale, between 0.5-4mm. Dry fractionation diagram combining grinding (based on shearing solicitation) and separation step (based on size and density) have been developed at the kilogram scale in order to separate the rind from the pith of maize stem internode while preserving pith alveolar structure. The fractions with particle size higher than 1 mm, contain more than 92 % of pith and represent 41% of the initial pith. In addition, in the finest fractions (particle size < 1mm), the pith vascular bundles are dissociated from parenchyma cells, and a successful isolation of the vascular bunds has been realized using an electrostatic separator. The fractions containing the biggest pith particles were retained to produce thermal insulating materials, which present a thermal conductivity around 0.04 W/mK, close of those of commercial thermal insulating materials.

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Oral communication

Extraction and Recovery of Sinapic Acid from Oleaginous Biomass (Mustard Bran): a Sustainable Access to a Valuable Phenolic Platform Molecule

Ezinne Achinivu, Erika Clavijo Rivera, Amandine Flourat, Florent Allais

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The objective of this research project was to develop a cost-effective and sustainable integrated process for the extraction and recovery of sinapic acid from oleaginous “oil-bearing” biomass as a value added chemical alongside cellulose and lignin. Waste products like mustard bran and canola meal are rich in sinapine (~15 mg/g), a derivative of sinapic acid, which has the potential to serve as a value-added product for applications in the cosmetic, the plastic and the pharmaceutical industries due to its antioxidant and anti-UV properties.

By employing an optimized chemo-enzymatic technique, sinapine was effectively liberated and completely converted into sinapic acid. Following this, the sinapic acid was recovered using an extensively optimized membrane filtration technology involving nanofiltration and diafiltration. Lastly, the residual biomass (fats, cellulose, lignin) were also fractionated and characterized for their use in other products. With these unique approaches in fractionation, separation and process integration, the process developed has an improved efficiency, cost effectiveness and environmental impact. The establishment of these processes can lead to new technology developments, and economic opportunities, which can, in turn, enable the cost-effective production of advanced bioproducts.

Invited lecture

Lignocellulose deconstruction and the importance this has on maximising the use of chemical functionality in bio-based platform molecules

Thomas J. Farmer

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The key to future successful biorefineries will be a combination of the ability to handle varied and diverse feedstocks and to also produce a wide range of products. In doing so the ambition will be to utilise all component parts of feedstock, but as we know the chemical composition of biomass is incredibly diverse. At the heart of these future biorefineries will be the production to a range of Platform Molecules, these being fundamental building blocks of a bio-economy. Although in essence platform molecules would seem analogous to fossil-derived base chemicals, they are in fact vary different [1]. For example, high heteroatom content of platform molecules means they contain significantly more functionality, this something we must embrace and not seek to remove. The platform molecules that can derived from your biomass are also determined by the feedstocks chemical composition, and most important of all how you process the feedstock. As such careful, selective, sequential deconstruction is required to maximise feedstock utilisation. This talk will highlight the value of the diverse chemical functionality of platform molecules [2]. It will also focus on how different components of lignocellulose lead to different products and therefore why we need effective, selective and controlled separation of lignocellulose to form platform molecules.

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Oral communication

Biorefinery strategies based on Room Temperature Ionic Liquids, hydrolases and their synergism with other pretreatments

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Biorefinery concept is built around sustainable strategies of lignocellulosic biomass (LCB) transformation. The economic viability includes the development of eco-responsible processes targeting the three major constitutive components of LCB: cellulose, hemicellulose and lignin. In this context, sequential and simultaneous strategies were developed including a rational selection of Room Temperature Ionic Liquids (RTILs) and (hemi)cellulolytic enzymes. These approaches were applied to representative LCBs including agricultural or forestry residues and dedicated crops.

Sequential strategy consisted in RTIL-pretreatment implemented in mild conditions (110 °C; 40 min) followed by enzymatic hydrolysis. Whatever the targeted LCB, pretreatment induced a significant improvement of cellulase-catalyzed hydrolysis performances. The glucose yields were 4 to 8 higher depending on biomass as compared to untreated [1-3]. Chemical composition and structural studies of pretreated LCBs evidenced a lignocellulosic matrix disorganization rather than fractioning. According to the crystallinity index variations induced by the pretreatment, the improvement of enzymatic saccharification cannot be exclusively linked to an increase in the amount of amorphous regions of cellulose. For wheat straw, the addition of one supplementary xylanase-catalyzed step in the sequential strategy led to efficient xylose production with very competitive yield (97.6%) and a subsequent total enzymatic saccharification of constitutive cellulose (glucose yield > 99%) [3]. The combination of this strategy with other pretreatments such as subcritical water or reactive extrusion is also investigated to evaluate their complementarity [4].

Otherwise, tolerance of cellulases and xylanases to RTIL was demonstrated. In view of reducing the RTIL amount and the number of steps, simultaneous strategy combining RTIL pretreatment and enzymatic hydrolyses in a one-batch process was proposed. This route provided promising sugar yields in comparison with the sequential strategy [5]. This effectiveness was suggested to be governed by a compromise between better substrate accessibility and enzyme deactivation.

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Oral communication

Innovative high-throughput microplate approach for cell wall degrading enzyme production based on fungi and lignocellulosic raw biomass interaction

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The industrial exploitation of lignocellulosic biomass for platform molecules production is currently facing two major bottlenecks [1]: the plant cell wall chemical composition is extremely complex and its degradation therefore requires the combination a large variety of enzymatic activities [2]existing methods for high-throughput screening of such cocktails are not applicable to complex substrates. We therefore propose an innovative high-throughput screening methodology for the rapid design of enzymatic cocktails, based on the interaction between fungi and biomass in a submerged manner and that can be applied to any kind of biomass without extensive pre-treatment. This approach, developed on the REALCAT platform, uses the BioLector® as core equipment. This device enables the screening of up to 48 fermentations of fungi/biomasses couples per batch, in a small-scale format, and is totally automated.

As proof of concept, we tested one plant pathogen, *Zymoseptoria tritici*, and two species well known for their ability to produce a wide range of cell wall degrading enzymes, *Aspergillus niger* and *Mucor circinelloides*, with wheat straw has complex model substrate [1–3]. The biomass was grinded before use to reduce the particle size, but no further treatment was applied. Cultivation was performed in a submerged mode by adding the minimal medium M3. In order to characterize the cocktails produced, fermentation supernatants were sampled over time, and enzymatic activities were determined using 7 different model substrates (AZO-xylan, AZO-cellulose, AZO-rhamnogalacturonan, AZO-barley glucan, AZO-casein, pNP-glucopyranoside and pNP-xylopyranoside) in a high-throughput automated colorimetric assay. Control fermentations were done in flasks, and results finally showed equivalent to higher enzymatic activity production with our new approach. With a much smaller volume (1.4 mL against 100 mL) and equivalent enzyme production, this automatable method proves to be very efficient for the high-throughput screening and design of fungal enzymatic cocktails toward a wide range of biomass complex substrates.

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Keynote lecture

Closing the loop between soils and lignocelluloses to address global challenges

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Soils are an essential resource for our societies and at the cross road of major challenges: food security, renewable energy provision, water security, biodiversity maintenance and climate change. Indeed, soils contain twice as much carbon as the atmosphere and could then contribute to mitigating climate change by storing more carbon in the form of soil organic matter. This has been recently emphasized by the 4p1000 initiative proposed at the COP21, which fosters the preservation and increase of the organic matter content of soils for food security, adaptation to and mitigation of climate change

Plant biomass is the main source of soil organic matter and lignocellulosic biomasses the main source of organic carbon to soils. Maintaining or increasing the inputs of plant biomass to soils is therefore crucial, at the local scale for soil fertility and biodiversity and other ecosystem services, and at the global scale for climate change mitigation. This can be achieved in agricultural soils through an increased primary production with adequate agricultural practices (e.g. cover crops, agroforestry), while ensuring sufficient plant residue return to the soil. Recent results show that increasing plant biomass inputs to soils is more effective to increase soil organic carbon stocks than no tillage practices.

How much biomass should be returned to soil to maintain or increase its carbon stocks and its fertility? What are the effects on soil fertility and soil carbon stocks of returning more or less processed biomass to soil (e.g. straw, compost, digestate..)? Where should the biomass be returned to soil in priority at the landscape scale? These are questions and challenges to be explored for an optimized management of lignocellulosic biomasses in a bioeconomy addressing global challenges.

Invited lecture

Lignocellulose in forest soils and its microbial decomposers

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Forest soils represent important terrestrial carbon (C) pools where C is primarily fixed in the plant-derived biomass [1]. This is why lignocellulose represents the most important complex source of organic matter in this environment that is typically utilized by fungi and bacteria. Both fungi and bacteria are involved in the assimilation and mineralization of C from lignocellulose as well as other major complex sources existing in soil. Decomposer fungi are, however, better suited to utilize lignocellulose, whereas the ability to utilize fungal and bacterial biomass is more frequent among bacteria [2]. The enzymatic systems for lignoellulose decomposition in forest soils are complex and highly redundant and individual families of decomposer genes are typically produced by hundreds or even thousands of microorganisms. While fungi seem to dominate the pool of transcribed ligninolytic enzymes, bacteria have an equal importance in the production of cellulases and hemicellulases [3]. Indeed, the ability to decompose cellulose and other plant polysaccharides is common among bacteria inhabiting plant litter and the organic layer of soil. Interestingly, the enzymatic systems of bacteria can be highly variable in composition, ranging from simple to very complex ones [4] and give some of their producers the ability to efficiently decompose even such complex compounds as crystalline cellulose [5]. It appears that lignocellulose decomposition in forest soil is a collaborative process where a wide range of microbial taxa collaborate and this environment still represents a largely unexplored source of biotechnologically relevant strains.

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Oral communication

Soil microbial response to sunflower residue and corresponding agromaterial inputs

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Industry, transportation or building sectors consume fossil fuels and generate waste and pollution. The use of 100% biobased materials in these fields is an ecological alternative to fossil materials and prevents resources depletion [1]. Nevertheless, the end-of-life issue of these agromaterials is to be considered in waste management.

In this context, we imagined a biological recycling of an agromaterial made for the building sector. Indeed, agricultural soils provide many services, such as providing food and materials for human needs. They can also contribute to the greenhouse gases mitigation through carbon storage following organic matter incorporation (organic waste, crop residues) [2].

In this study, we separated sunflower stems into: the bark (outer stem part) and the pith (inner stem part), then used pith for designing the building agromaterial. Our aim was to compare soil response when we introduce the pith and its corresponding agromaterial through carbon and nitrogen dynamics and microbial community monitoring.

Various methods were used to characterize both biomasses. The decomposition study was inspired by the French standard XP U44-163 [3]. It was conducted in microcosms under controlled conditions (28 C° and 83 incubation days). Carbon and nitrogen mineralization were monitored at several dates. For microbial communities dynamics, various measurements were carried out (microbial carbon, total DNA, bacterial DNA, fungal DNA and B-Glucosidase) on 5 incubation dates (0, 8, 21, 49 and 83 days).

The agromaterial presented higher mineralization rates than the pith. However, the measured biological indicators seemed little affected by the biomass type. Our results suggest that the manufacturing process may have induced some biochemical modifications of the original material, favoring the C mineralization, but not enough for observing important microbial differences. The decomposition rate of the pith agromaterial also shows that the biological recycling we are proposing may be a suitable way of waste management.

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Oral communication

Microbial colonization of hemp mulches during field retting at the soil surface

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The use of plant fibers as hemp (*Cannabis sativa* L.) has spread to new applications as substitute of synthetic fibers. The field retting, a process during which hemp stems are displayed at the soil surface after harvest in mulches to be exposed to heat, soil humidity and microorganisms, is an economical and widely used natural pre-treatment in Europe. Retting consists in a selective microbial degradation of the bast plant tissues surrounding the fibers which aims to facilitate their separation. This process is regulated by environmental factors, such as local weather conditions and soil microbial colonization. However, most available information is still mostly empirical. Improving the understanding of plant characteristics, microbial colonization and environmental conditions influence on retting is essential for improving farmer's retting management.

The aim of this work was to study the relative importance of retting drivers. An experimental approach under controlled environmental conditions was set up to mimic agricultural and climatic scenario of retting. This experimental design allows to decoupling the environmental parameters and thereby avoiding confounding effects, so as to quantify the effects of their changes on the retting dynamics. The impact of crop harvest stage (flowering and maturity of the seed) on retting, was assessed through the microbial colonization dynamics of the stem surface and the bast tissues degradation using infrared spectroscopy and colorimetry. It revealed the progressive microbial colonization and degradation, revealed also by wet chemistry, microscopic analysis, and linked with enzymatic activities. Topsoil analyses also showed microbial activities and nutrient exchanges during the process. Retting of stems harvested at flowering stage was faster than that of stems harvested at the seed maturity stage. The experimental design proved to be adapted to carry-out hemp retting with other scenarii varying climatic conditions, soils and hemp characteristics, while quantifying of the induced effects on the retting dynamic.

Keynote lecture

Zambezi Biorefinery: "Pure" glucose from 2nd generation feedstocks

Ed de Jong

Avantium, Amsterdam, the Netherlands;

Avantium (www.avantium.com) is a high tech SME company known of their exploration into novel furan (YXY) chemistry, focused on efficient and low cost conversion of C6 sugars via HMF derivatives into the promising chemical key intermediate FDCA. FDCA can be used as building block for a wide range of applications including polyesters such as PEF, polyamides, resins and plasticizers [1]. Currently, Avantium is working to bring 100% biobased PEF bottles to the market and intends to commercialize the YXY process in a Joint Venture together with BASF.

Many chemical building blocks can be produced from biomass, nowadays mainly from 1st generation based carbohydrates [2] but in the longer term brand-owners want to have the option to choose between 1st and 2nd generation feedstocks. The use of non-edible lignocellulosic feedstocks is an equally attractive source to produce chemical intermediates and an important part of the solution addressing these global issues (Paris targets). Avantium's strategic objective is to deliver with it's 2nd generation Zambezi technology the best in class 2G "pure" glucose technology for (bio-)chemical & bioenergy applications for a sustainable future; in parallel delivering value generation from the implementation of this technology. All products streams should be marketed at their highest value [3]. In this presentation particular attention will be given to the Zambezi technology for the production "pure" 2nd generation glucose, hemicellulose streams and lignin. Avantium has achieved a range of technological improvements on the concentrated mineral acid based process to make the process techno-economic competitive. A consortium consisting of AkzoNobel, RWE, Chemport Europe and Staatsbosbeheer has been established to bring this technology to commercial scale. As a first step a demonstration plant is being built in Delfzijl, the Netherlands to be operational in mid-2018.

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Invited lecture

Levoglucosenone: à la recherche de chimie perdue

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Levoglucosenone ($C_6H_6O_3$ – CAS No. 37112-31-5) is arguably the most valuable low molecular weight compound that can be produced by relatively simple processing of unrefined lignocellulose. First identified in the early 1970s [1,2] in the volatiles emitted by pyrolysing paper that had been treated with acidic fire retardants, levoglucosenone has taken 40 years to come into semi-commercial production [3]. The high reactivity of levoglucosenone, particularly towards the reactive chars that are produced as a by-product of lignocellulose pyrolysis, meant that all attempts to scale up the production of levoglucosenone beyond a few grams per day failed. Levoglucosenone consequently languished as a 'laboratory curiosity' for several decades. In 2009, the small Australian chemical company, Circa Group, rediscovered the chemical potential of levoglucosenone and set about developing a continuous catalytic flash pyrolysis process that would enable the chemical properties of levoglucosenone and its chemical derivatives to be exploited on a multi-tonne scale. Working initially with only three full-time research staff and minimal private investment capital, Circa has successfully developed the first continuous process for making levoglucosenone and its dihydro-derivative, trademarked as Cyrene®. Through collaboration with the groups of Prof. James Clark at the University of York [4], Prof Florent Allais at AgroParisTech [5] and at several Australian and UK universities [6-8], Circa has been able to demonstrate that Cyrene® has a potential market of many thousands of tonnes, primarily as a replacement for two toxic polar aprotic solvents, NMP and DMF, and also as a valuable chiral bio-based chemical intermediate. This lecture will describe how, by setting seven important boundary conditions on their R&D, Circa Group successfully developed using minimal resources the first continuous process for producing levoglucosenone to the point where Circa Group has now formed a joint venture with pulp and paper company, Norske Skog (Australasia), to build and operate the world's first large-scale demonstration plant that will produce 50 tonnes per year of Cyrene® and plans to build a 5,000 tonne per year commercial plant in 2019-20. The unusual physicochemical and toxicological properties of levoglucosenone and Cyrene will also be described.

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Oral communication

New approach toward lignin conversion to fuel and chemicals

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This presentation will discuss the development of a simultaneous lignin-to-fuel and chemicals conversion process which can be integrated into a biochemical based lignocellulosic biorefinery process.

Oral communication

Hydrothermal conversion of lignocellulosic sugars to furans

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Carbohydrates as the most abundant renewable resource represent an important basis for sustainable future production technologies. The joint project BBCHEM aims to develop the conversion of lignocellulosic raw materials to bio-based chemicals with the help of hydrothermal conversion processes. Based on the principal constituents of structural carbohydrates, i.e. glucose and xylose, the production of the target chemicals furfural and 5-hydroxymethylfurfural is investigated. Within the project, batch and continuous tubular reactors under high pressure and temperature were used to optimize carbohydrate conversion rates and product yields. A remarkable linkage of reactor setup and process parameters on carbohydrate conversion, target chemicals yields and side products (insoluble humins and organic acids) was found. Homogeneous acidic catalysts were found to significantly affect conversion and yields. In co-conversion of C5 and C6 sugars, similar conversion was achieved pointing at a combined use of lignocellulosic carbohydrates within hydrothermal liquefaction. Challenges were found to be the decomposition of target chemicals and associated humins formation. The platform chemicals obtained are potential candidates for applications e.g. in bio-based thermoplasts and thermosets, as fuel additives or for solvents production.

Oral communication

Enzymatically-synthesized alkyl pentosides and pentose-based esters, as surfactants of interest

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Xylans are abundant polysaccharides in lignocellulosic biomass and their valorisation represents a key issue for the development of biorefineries. Xylans and their derivatives provide a large spectrum of applications in biorefineries, such as the production of fermentation products (ethanol, xylitol), prebiotics, or films for packaging materials [1]. Finding new applications for pentose-based molecules is still of interest and challenging for the valorisation of lignocellulosic biomass.

In this context, our research is devoted to the enzymatic functionalization of pentoses into surfactants. Enzymatic pathways for the functionalization of sugars gain interest as they occur in one step with high selectivity. We developed a first approach dedicated to transglycosylation reactions catalysed by hemicellulases in the presence of alcohols. This allowed producing various alkyl pentosides and oligopentosides from xylans [2, 3]. In another approach, we developed the synthesis of pentose-based esters with lipases in presence of xylose, arabinose or xylo-oligosaccharides and esterified fatty acids [4]. Synthesis of alkyl pentosides and pentose-based esters was also developed directly from lignocellulosic biomass such as wheat bran. Our results indicate that both alkyl pentosides and pentose-based esters exhibit interesting surfactant properties for applications as ingredients for detergency, cosmetics, ... Furthermore, these non-ionic bio-based surfactants are not toxic and are biodegradable. These studies are currently developed in the European Interreg ValBran project.

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Oral communication

From sawdust to high value-added fine chemicals: a France-Australia-USA success story

Louis Mouterde[1], Aurélien Peru[1], Amandine Flourat[1], Louis Mouterde[1,2], Andreia Teixeira[1], Christian Gunawan[3], Warwick Raverty[3], Martyn Jevric[4], Jon Stewart[2], Ben Greatrex[4], Florent Allais[1]

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Levoglucosenone (**LGO**), an renewable enantiomerically pure α,β-unsaturated ketone, can be efficiently produced from cellulosic byproducts by the Furacell process™ developed by CIRCA Group [1]. This highly dehydrated sugar has been used previously in the preparation of chiral synthons such as (-)-γ-multistriatin and (+)-Prelog-Djerassi lactonic acid.

Interestingly Baeyer-Villiger reaction on levoglucosenone and its dihydro derivative (aka **2H-LGO** or Cyrene®) provides (S)-γ-hydroxymethyl-α,β-butenolide (**HBO**) and (S)-γ-hydroxymethyl-α,β-butyrolactone (**2H-HBO**), respectively, which are valuable chiral chemical platforms suited for the synthesis of polymers, drugs, flavors and antiviral agents to name a few. Nevertheless, classical Baeyer-Villiger oxidation uses harmful peroxides such as *m*-chloroperoxybenzoic acid (*m*-CPBA), thus limiting its suitability for the production of **HBO** at the industrial scale.

Herein, we report sustainable and efficient synthetic processes allowing the multi-kilo scale production of **HBO** and **2H-HBO** using organic solvent- and catalyst-free Baeyer-Villiger oxidation [2], lipase-mediated Baeyer-Villiger oxidation [3] and whole cells bioconversion [4]. The chemo-enzymatic transformations of **HBO** and **2H-HBO** into valuable chiral fine chemicals such as flavors, chiral epoxides [5] and rare sugars will also be described.

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Keynote lecture

High Performance Biobased Structural Epoxy Resins Derived from Levulinic Acid

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Our group developed a series of biobased epoxy resins from readily renewable building blocks that have tunable properties for a range of applications. One route builds from levulinic acid a series of diglycidal ether diphenolate esters (DGEDP-esters). The ester group provides a simple approach to tune epoxy monomer viscosity as well as cured resin properties [1]. Combinations of DGEDP-methyl ester with a biobased epoxy cardanol resin provide an approach to dramatically enhance cured epoxy resin toughness relative to either of the neat resins [2]. The properties of DGEDP-pentyl ester was expanded by its combination with the monofunctional glycidyl ether of eugenol (GE) that functioned as a reactive diluent [3]. Using this reactant diluent to adjust the viscosity and cure time, the resins were utilized in a vacuum infusion molding process with plain woven glass fiber mats to fabricate a glass fiber/epoxy resin composite [4]. In addition, studies were performed using Bacterial Cellulose as a reinforcement matrix.
Given the growing importance of structural capacitors, the potential replacement of DGEBA structural dielectric materials with DGEDP-esters (i.e. methyl, ethyl, propyl and butyl esters) was studied. Broadband dielectric spectroscopy revealed that DGEDP-propyl has the highest dielectric constant in the series, comparable to DGEBA. Differences in the dielectric properties of DGEDP-esters is attributed to the interplay of segmental, small local, and side-chain motions on one hand and free volume and steric hindrance on the other. The introduction of surface amine functionality to cellulose nanocrystal allowed for the preparation of DGEDP-ester nanocomposites [5]. The resulting thermomechanical properties of epoxy nanocomposites with amine functionalized cellulose nanocrystal were 7 times greater than nanocomposites prepared using unfunctionalized cellulose nanocrystals. Finally, the use of DGEDP-esters as components in structural adhesives was studied. The components consisted of a DGEDP ester, bis(furfurylamine) and modified cellulose nanocrystals. By manipulation of the structures and formulation composition, we obtained a biobased structural adhesive with suitably high viscosity, decreased cure time and adhesion that competes with commercial adhesive systems. The thermomechanical properties of these materials determined by DSC, DMA, tensile testing, fracture toughness will be discussed. Control of epoxy resin viscosity enabled the use of selected epoxy resin formulations discussed above for the preparation of fiber infused epoxy resin materials.

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Invited lecture

Iso-Lignin® Technology, Lignin base polyurethane: challenges and opportunities

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Lignin is the most abundant aromatic biopolymer in the world and the second most abundant natural polymer after cellulose. Lignin is a complex bio-polyol which is extracted from the non-food grade biomass, such as vegetable straw and wood. Due to its high molecular weight and its complex structure, those hydroxyl functional groups are not easily accessible.

The Iso-Lignin® Technology is a patented process to make lignin-based polyurethane products comprising:

- rigid foams used in buildings, transportation, packaging and appliance;

- flexible foams used in furniture and bedding;

- coating and adhesive;

- structural board…

The lignin is first mixed with the isocyanate, a basic raw material used in the chemistry of polyurethane products. This pre-polymer is then further reacting by using catalysts, heat or a resin to achieve the final product. The process does not require the use of any solvent, nor chemical derivatization of the as-obtained lignin. Consequently, any source of lignin can be used in the Iso-Lignin® Technology. No objectionable odor due to lignin has been detected, thus opening a wide range of applications.

This presentation will present an overview of the work performed to overcome the lack of standards and to accelerate the industrial development and the commercialisation of biobased polyurethanes.

Oral communication

Lignin cross-linking in cellulose nanocrystal based films by Fenton reaction: mechanism and properties

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Nowadays, the conversion of lignin residue into renewable chemicals is one of the greatest challenges in developing economically viable industries based of lignocellulosic biomass. With their phenolic and complex structures, lignins are under valorised to new commercial products of interest partly from their high sensitivity to oxidation and to uncontrolled affinity with other molecules. The most investigation application today is its natural function to glue and hold together the cellulose microfibrils in plant cell wall and in wood panel [1]. Concomitantly, the investigation on cellulose-based nanocomposites associated with other plant polymers for new functionalities increased considerably due to the range of applications of such high added-value nanomaterial [2].

Under controlled conditions, Fenton reagent has been used to grafted lignin onto cellulose nanocrystals surfaces to form coatings or films with interested functional properties (UV-blocking, water resistance, antioxidant) [3,4]. Fenton reagent is defined as a mixture of hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) and it is one of the most powerful oxidative system found in nature producing highly reactive hydroxyl radicals [5].

In this context, the mechanism of Fenton reaction was studied in the objective to control the final structure and properties of film. Two strategies were investigated: first, the Fenton reagent (Fe^{2+}/H_2O_2) was studied to measure the production of hydroxyl radicals and their effect on cellulose colloidal suspension in presence of monomer of lignin. Second, coatings and films were prepared from these mixtures to analyse the impact of the oxidative reaction on cohesiveness of polymers in the material. Several analytical methods were developed to identify the complexes in solution and in the film. In addition, a thorough study of radicals in each film was carried out by EPR. Finally, the water resistance and anti-oxidant properties, as interesting target functionalities, were determined for the same films.

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Oral communication

Valorization of lignocellulosic fibers derived from Park and Garden wastes for Biocomposites applications

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Biocomposites based on lignocellulosic raw materials are a solution to the current demands in terms of valorization of lignocellulosic solid waste, reduction of our dependence on fossil resources, reduction of environmental pollution generated by non-biodegradable and / or non-recyclable plastics, and eco-design of high-performance materials. The use of microbial biopolyesters such as polyhydroxyalkanoates (PHAs) as a polymer matrix, which can also be produced from waste, allows us to obtain fully biobased and biodegradable biocomposites [1-2].

Today, the two main ways of recycling green waste from parks and urban gardens are composting and anaerobic digestion. In our study, we assessed the potential reinforcing effect of this lignocellulosic biomass. This resource is a very heterogeneous material; it consists mainly in organic (such as leaves, branches or grass clippings) and inorganic (soil and stones) compounds, and also includes foreign objects such as plastic bags, glasses or papers. In order to understand the overall reinforcing effect while taking into account this heterogeneity, the raw material was sorted in such a way to evaluate the respective reinforcing effect of each main fraction (wood-branches, leaves and grasses). The production of reinforcing fillers was carried out exclusively by dry fractionation, which consists in a combination of sorting and grinding processes.

Six fractions were identified:
- Unusable Fraction containing soil, stones and foreign objects (13wt%),
- Branches Fraction (23wt%),
- Leaves Fraction (5wt%),
- Grass Fraction (3wt%),
- A Medium Fraction consisting in a mixture of leaves, branches and grass without soil (9wt%),
- A Fine Fraction is similar to the Medium Fraction with soil (47wt%).

The reinforcing effect of each fraction was evaluated and discussed in relation to the structure of PHA-based biocomposites materials. This study is part of the European RESURBIS project (http://www.resurbis.eu, 2017-2020) aiming at enhancing the value of all urban organic waste.

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Oral communication

Water-Assisted Extrusion Compounding Process to Reduce the Total Volatile Organic Compounds in Natural Fibre-Filled Composites for Automotive Interior Applications

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Natural fibre-filled composites are recently considered to be used in interior parts of vehicles because of their eco-friendly and mechanical performance [1-3]. However, they emit VOCs at all stages of their life cycle, such as compounding, injection-moulding and usage [4]. In the present study water-assisted extrusion compounding process has been used to reduce the VOC emission during the usage of injection-moulded parts. Flax and hemp fibres (20 wt.%) reinforced polypropylene composites were compounded in presence of maleic anhydride grafted polypropylene (PP-g-MA) as compatibilizer using twin screw extrusion, with and without water injection during extrusion. Then the compounds where injection-moulded into standard test specimen. Physical and mechanical properties such as morphological, fibre length, tensile and impact properties were characterized as well as the total volatile organic compounds (TVOCs) and odour emission using automotive standard D42 3109-C. Released TVOCs from the composite products were quantified by air sampling on adsorbent followed by thermal desorption and GC-MS analysis. The scanning electron microscopic observation indicated a good dispersion of fibres in the matrix with a low reduction in average length and aspect ratio of fibres. Mechanical properties of samples produced with water-assisted compounding showed slightly reduced modulus and strength compared to the samples prepared without water assistance. For both flax and hemp fibre-reinforced composites, TVOC emissions are reduced by 94% and 30% respectively with water-assisted extrusion. So, the water-assisted extrusion process has proved its effectiveness in reducing VOCs emissions without scarifying the mechanical properties.

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Oral communication

GuyaValoFibres: exploring the potential of amazonian unvalorized ligno-cellulosic resources for fibres-based composites applications

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Demand for technical and sustainable building materials is a hot topic in tropical areas. This is why in these regions, a special focus has recently been laid on the development of local biobased products. To encourage and foment this movement towards, we built a project in French Guiana that aims at valorising ligno-cellulosic fibres from Amazonian resources as reinforcement in composites materials, taking advantage of the good mechanical properties of their fibres. Indeed, first experiences in surrounding countries evidenced the interest of several plant species for composites applications [1-3]. In this project, we plan to enlarge the number of studied species with a focus on ligno-cellulosic resources that remain non-valuated today: tree species unsuitable for traditional timber productive systems, residuals from sawmills or agricultural wastes. In addition, exploring local high biodiversity, we expect to identify high-value fibres originating from untapped or uncharacterized plant species. GuyaValoFibres project´s objective is to add value to local ligno-cellulosic resources in the form of structural and insulating fibreboards, in the view to motivate the future development of a secondary productive chain in French Guiana dedicated to the production of biocomposites. This presentation focuses on the description of six different identified resources of interest and their fibres properties. Combining technics developed for paper and fibres science [4], we propose an innovative way to isolate fibres bundles and characterize their morphology, water uptake, mechanical properties and bonding ability [5]. From these results it will be possible to orientate (i) the choice of the matrix and the processing conditions that should be used for the elaboration of thermoplastic biocomposites by extrusion and (ii) the conditions and parameters that should be used in the process of fibreboards production.

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Oral communication

Quality management of hemp straw used in agro-materials

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First producer in Europe, France has a large industrial potential in valorization of hemp. Its fibers constitute a renewable and ecological resource, with high potential uses in innovative materials with high added value. Nowadays mainly used in the paper industry, the hemp fibers have exceptional properties sought for technical applications (thermo compression, thermoplastic composites,) which attract many sectors of application, such as transport, textile industry, etc. However, access to those markets involves i. to produce fibers having strict specifications and ii. to be able ensure the required quality. The project, RIGHTLAB, aims to identify these fiber quality indicators.

For these markets, the hemp straws, from which the fibers are extracted, have to undergo a specific stage, called retting. Usually, this bioprocess consists in laying the stems on the field during several weeks after their cutting. This step will cause several transformations of the straw and hence of the fibers. Owing to its methodology, the retting remains difficult to control, and its estimate, i.e. the degree of retting, is empiric and subjective. Thus, the lack of method to measure the degree of retting is greatly detrimental to the development of the sector and to the hemp valorization with high added value.

Led by La Chanvrière, the project aims to define indicators of the retting degree. The strategy put in place starts in the fields by the characterization of the retted straws using spectrometric, chemical and mechanical methods and finishes with the analyze of nonwoven and injected thermoplastic composites made of fibers extracted from these straws.

Exploring lignocellulosic biomass!
Challenges and opportunities for bioeconomy
26 - 29 June 2018 in Reims - France

Poster Abstracts

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Biorefining for Bioeconomy

Session 2
Structural and chemical complexity of lignocellulose

Session 3
Physical, chemical and biological deconstruction of lignocellulose

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Poster 1

Total synthesis of lignin model compounds: dimers of sinapylic alcohol (S) and dimers and trimers of coniferylic alcohol (G)

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In order to further the understanding of lignin formation and more importantly lignin depolymerisation, employed synthetic model compounds have to be able to match the properties of their natural lignin counterparts as closely as possible. To fulfil this prerequisite, we propose a convenient chemo-enzymatic synthesis for natural-occurring dimers of coniferylic alcohol (aka monolignol G), sinapylic alcohol (aka monolignol S) as well as for a trimers and its dehydrotrimer (β-5)-(β-*O*-4) of monolignol G [1]. For the dimers β-5 and 5-5 of monolignol G, linkages were obtained through radical coupling catalyzed by laccase. The yields achieve 43% and 95% respectively. Whereas, the ether bond β-*O*-4 was formed by SN2 between a phenolate and a bromo ceto ester (83%) or by aldolisation (78%) [2]. Dehydrotrimer and trimer were synthesized from ferulic acid in nine steps with a global yield of 20% and 12% respectively. For dimers of sinapylic alcohol, optimization of the reaction conditions (i.e. temperature, enzyme addition, solvent ratio) lead to an inversion of selectivity between the two dimers β-*O*-4 and β-β [3].

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Poster 2

Analytical characterization of a soluble lignin fraction from thermomechanical softwood treatment: toward a biorefinery concept

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Valorization of by-products from paper industries produced with chemical pulp is a well-known economical and technical scheme. It is not yet the case for by-products coming from thermomechanical pulp (TMP). TMP process waters contain a wide range of valuable compounds as lignin and phenolic extractives. Thanks to a membrane filtration process (ultrafiltration and nanofiltration), previously developed in the laboratory, soluble lignin extracted by TMP was isolated and concentrated in a liquid fraction. The following data resume its chemical and structural characterisation needed for selecting its potential valorisation pathways.

Chemical composition of the fraction was depicted after sulfuric acid hydrolysis as follow: around 30 % of lignin (acid soluble lignin measured by UV-spectrophotometer at 205nm and acid insoluble lignin by gravimetry) and 60 % of sugars (HPAEC-PAD) corresponding exclusively to hemicelluloses and more particularly to galactoglucommanans[1]. Size exclusion chromatography in 10mM NaOH showed that the lignin fragments extracted are relatively light with a molecular weight distribution centered around 6 to 8 kDa. Superposition of the signals from UV and RID detectors suggests covalent linkages between sugars and lignin. 2D HSQC RMN spectrum confirmed the presence of covalent linkages, corresponding mainly to phenyl-glucoside bonds[2].

Considering the fact that lignin is not soluble in water and that galactoglucomannans can be solubilized in water after 4 h at 80 °C[3], hot water extraction was performed to quantify the ratio of isolated lignin vs lignin-carbohydrate complexes (LCC). This experiment showed that a large majority of the TMP lignin, exactly 77.1%, is connected with hemicelluloses and thus referred as LLC.

LCC have been reported in the literature to present interesting biological properties such as anti-UV and anti-scavenger effects[4]. This lignin soluble fraction from TMP biorefinery may then have a good potential for cosmetic applications with high added values.

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Poster 3

Spatio-temporal imaging of lignocellulosic biomass deconstruction and correlation with the enzymatic hydrolysis

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Lignocellulosic biomass (LB) is a renewable resource from plants used as an alternative to fossil resources [1]. Bioenergy, biomaterials and biomolecules could be produced based on LB without compromising global food security. However, LB is recalcitrant to enzymatic deconstruction due to its chemical composition and its structural complexity [2]. The major reason is the cross-linking between polysaccharides and lignin making a barrier to wall degrading enzymes. Different chemical and physical features have been proposed to explain LB recalcitrance and to predict its deconstruction [4]. However none of them seems to be universal but rather specific to biomass species and pretreatment. One key progress is to achieve a better understanding of the evolution of the 3D architecture of LB during the enzymatic hydrolysis through 4D imaging.

The aim of our project is to propose a novel approach to monitor the dynamics of enzymatic deconstruction of LB using fluorescence confocal microscopy, image analysis and to identify some structural features which can explain and predict and optimize the process of hydrolysis.

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Poster 4

Following sugar release and particle size evolution during enzymatic saccharification reveals distinct degradation patterns of maize lignocellulose fractions obtained by dry fractionation

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Over the last 10 years, research efforts have been dedicated to identifying the factors that influence lignocellulose enzymatic deconstruction. No consensus has been reached except for the detrimental role of lignins. This may be ascribed to the heterogeneity of plant biomass. To take into account this parameter, dry fractionation was applied to maize biomass. Separation process was first based on particle size by air classification, and then on sample composition by electrostatic separation. Six fractions were obtained and characterized, for their physicochemical characteristics, and saccharification capacity. Saccharification was carried out using a torus reactor allowing the analysis of the saccharification yield coupled to the real time monitoring of changes in particle size [1].

Fine, medium and coarse fractions accounted for 43%, 22% and 35% of the initial biomass and showed a median particle size around to 60, 170, and 310-µm, respectively. Electrostatic separation did not lead to further separation according to particle size. Fractions mainly differed by their amount of minor sugars and by the ratio syringyl to guaiacyl lignin units. Saccharification was evaluated by combining the hydrolysis yield and particle size reduction. In general, the release of sugars was correlated with particle size decrease. Interestingly, one of the medium size particle fractions behaved differently: high sugar release was observed without any significant change in particle size. This specific behavior was not associated with specific physicochemical properties. Looking at specific surface, a global negative correlation between particle size and specific surface suggested that the surface accessible to enzyme is highly related to geometrical parameters of maize stem particles with few impact of open porosity at mesoscale.

The present work demonstrates that different enzymatic degradation pattern can be revealed within a plant, following both changes in particle size and sugar release. Further work is needed to interpret the differences observed between maize sub-fractions.

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Poster 5

Lignin depolymerization by lignolytic enzymes/mediator system

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The valorization of lignin is a key element of the economic sustainability of 2nd generation biorefinery as it is the main renewable raw material composed of aromatic patterns that is present in the lignocellulosic biomass. Currently, this natural polymer is mainly valorized by combustion for energy production. A new promising way is the controlled depolymerization to obtain aromatic monomers or oligomers.

The aromatic compounds known as BTX (benzene, toluene, xylene) are the basis of today petrochemistry. Their substitution with lignin-derived monomers would open up a high value valorization for lignin. In addition to the physical and chemical methods reported in literature, the enzymatic approach has several advantages: mild conditions, reduced energy consumption, limited waste production and higher molecular purity.

In order to design a biological depolymerization process, we sought to create a screening method to elaborate an enzymatic cocktail to promote the depolymerization of lignin, the latter aiming at reducing the size of lignin oligomers and at releasing phenolic monomers.

To do so, a set of enzymes with commercial lignolytic activities and derived from fungal secretomes (produced *in situ*) has been selected. In addition, we have developed different analytic methods to monitor the depolymerization of lignin and the production of phenolic monomers during this lysis: chromatographic methods (HPLC, GPC and GC-MS) and a spectrophotometric method based on the method Folin-Ciocalteu. Conventional methods of enzyme assays (laccase, peroxidase, oxidase, etc.) have also been used.

The combination of these enzymes with chemical mediators show interesting results in lignin depolymerization.

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Poster 6

Improvement of protein content and limitation of polyphenolic rate in Olive Cake by solid-state fermentation: a way to valorize this industrial by-product in animal feed

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My abstract deals with the bioconversion of the olive cake (OC) generated by the olive oil industries in Morocco using selected filamentous fungi in solid state fermentation to upgrade its nutritional values for its subsequent valorization as ruminants feed. Four fungi were cultured on OC for 15 days. Chemical composition as well as enzymes activities were determined. The results obtained showed (i) an increase in the protein content for treated OC and (ii) significant ($P < 0.05$) decreases in the values of phenolic compounds. Moreover, the RP-HPLC analysis of OC confirmed the degradation of individual phenolic components by the strains. The present findings revealed 2 strains to be efficient organisms for their enzymes production and simultaneous enhancement in nutritive value of this by-product.

Poster 7

The phyllosphere as a new niche of lignocellulolytic microorganisms

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The phyllosphere, which is defined as the aerial parts of plants, is one of the most prevalent microbial habitats on earth (up to 10^{26} bacterial cells for the global phyllosphere) [1]. The microorganisms present on the phyllosphere can have several interactions with the plant. Indeed, plants will provide the habitat and several carbon sources (in low concentrations) to the microorganisms; on the other hand, microorganisms can promote the growth of plants [2]. Other neutral or pathogenic interactions could exist between the two biota [3]. The phyllosphere represents then a unique niche where microorganisms have evolved through time in that stressful environment and may have acquired the ability to degrade lignocellulose in order to survive to oligotrophic conditions.

In order to confirm this hypothesis, the dynamic lignocellulolytic potential of two phyllospheric microbial consortia (wheat straw and wheat bran) has been studied. These both agricultural co-products were harvested, ground (0.5-2 mm) and stored with % humidity < 10%. Growth of microorganisms was initiated by incubating the wheat straw and bran with minimal culture media at 30°C. The results showed a superior growth of the microbial community from the wheat bran than the straw and exhibited through their growth lignolytic (up to 116 mUI/mL) and hemicellulolytic activities (up to 220 mUI/mL) after 48h of growth. The microbial community at the end of the experiment was dominated by bacterial strains which some of them were isolated. Based on their morphological properties, 8 strains were isolated from each bran and straw consortia; all of the isolated strains which exhibited lignocellulolytic activities had a higher production of extracellular activities rather than intracellular; moreover, their hemicellulolytic potential was always higher than the lignolytic one. The microbial diversity of the bran and straw phyllospheres will be investigated by further omic approach in order to describe which microorganisms developed preferentially on bran or straw and characterize their potential lignocellulotyic function.

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Poster 8

Performance of a Stoichiokinetic model to predict the degradation of Lignocellulose by reactive oxygen species and the formation of valuable compounds

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The production of ROS (reactive oxygen species) can be an important step during the microbial degradation of lignocelluloses, since it promotes the effect of deconstruction enzymes on the plant cell walls [1]. ROS can also be used to functionalize the nanocelluloses by lignin-type compounds, or by their derivatives, thus opening an interesting way to obtain high added value nanostructured [2] objects as: emulsifiers, films and coatings with specific properties, resins for 3D printing ... ROS can be formed either directly by ferrous salts and/or hydrogen peroxide (Fenton reactions), or by the indirect action of the metalloenzymes, like Cellobiose DeHydrogenase (CDH) [3], involved in the biodegradation process.

The Lignoxyl project aims at identifying the active oxygen species involved in the oxidation of lignified plant walls, and to model the stages of their formation and of their reactions with lignocelluloses, by using polymer mixtures of increasing complexity (cellobiose, monomer (coniferyl alcohol) and oligomer of lignin, cellulose). The mathematical modelling of these reaction kinetics enables both: (1) to test the relevance of the proposed reaction schemes, and (2) to optimize the formation of the high added value compounds. A stoichiokinetic model has been used to predict the effects, either of the Fenton reactions, or of the CDH, on the formation of the products issued from the cellulose degradation. The first results prove that the model is able to predict the chemical kinetics observed experimentally, providing that the constant rate of the key reaction steps are identified, and that the variation of the iron reactivity in the medium is taken into account. Further work is needed to predict the effect of ROS on the formation of the high added value compounds from a simple lignocellulosic mixture composed of both cellobiose and coniferyl alcohol.

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Poster 9

Development of lignocellulosic biomass adapted hemicellulases pretreatment

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The objective of the BABET-REAL5 project is to develop an alternative solution for the production of 2G ethanol, competitive at smaller industrial scale and therefore applicable to a large numbers of countries, rural areas and feedstocks such as sweet corn cob (SCC) and barley straw (BS). These substrates are recalcitrant to enzymatic hydrolysis and numerous factors such as the presence of hemicelluloses and lignins are known to be responsible of the biomass recalcitrance to fractionation. These later ones are known to impede the access of cellulases to the cellulose part by forming physical barriers. A physico-chemical pretreatment is thus necessary to improve enzymes efficiency. Another challenge is to hydrolyze both cellulose and xylans component from biomass and to further ferment glucose and xylose into ethanol.

In this context, our goal is to develop efficient hemicellulasic cocktails adapted to the lignocellulosic biomasses. A thermophilic and hemicellulolytic bacterium (*Thermobacillus xylanilyticus*) was used to develop performant hemicellulasic cocktails for improving xylose and also glucose release from different lignocellulosic biomasses by acting in combination with cellulases. For this, we first evaluate the enzyme productions of this bacterium while growing on SCC, BS and wheat bran. The enzymatic cocktails produced were used to perform hydrolysis of extruded SCC and extruded BSS at different substrate loading (low to high consistency). The performance of the hemicellulasic cocktails were benchmarked with commercial cocktails. We showed that *T. xylanilyticus* was able to produce complete hemicellulases cocktails (containing xylanase, arabinosidase, xylosidase and esterases activities). The composition of enzymatic activities produced depends on the biomass used showing an adaptation of the bacterium to the chemical composition of the lignocellulose. Our results demonstrate that *T. xylanilyticus* cocktails are efficient for pentoses release from lignocellulosic biomasses.

Poster 10

Deciphering controlled cell wall degradation during flax dew retting: exploring potential enzymatic activities by metatranscriptomics

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Flax (*Linum usitatissimum* L.) bast fibres, characterised by their richness in cellulose, are traditionally used to produce textiles (linen) or to reinforce composite polymers as an environmentally friendly alternative to glass fibres. Fibre qualities for both applications are influenced by fibre morphology and cell wall composition determined during plant growth, and modified to a greater or lesser extent during fibre extraction. The first step in the industrial transformation of flax stems into fibres is achieved by dew-retting on the soil surface. At harvest, the flax stems are 'pulled' (up-rooted) and laid down directly on the soil in swaths (strips). Subsequent exposure to alternating periods of rain and heat combined with the development and the action of soil microflora favour the separation of cellulosic bast fibres from the stems *via* the partial degradation of cell wall polymers. While recent studies have described the microbial dynamics that occur during this process [1-3], retting is still not fully understood and a more detailed description of the underlying enzymatic activities is required to identify potential biological and physico-chemical markers for a more efficient retting process.

In the framework of a collaborative French 'Future project' 'StructuratIoN de la filière Fibres techniques d'OrigiNe végétale pour usages matérIaux' (SINFONI) we have developed a metatranscriptomics approach coupled with metabarcoding to identify potential major enzymatic functions related to bacterial and fungal cell wall degradation. These approaches based on Next Generation Sequencing (Illumina Hiseq and MiSeq system) are allowing us to access the exogenous (soil) and endogenous (plant) enzymatic arsenal potentially involved in degrading cell wall polysaccharides during dew-retting in flax. A more complete characterization of the enzymatic and biological diversity during retting should enable us to obtain a better understanding of the morphological and chemical changes that the stem undergoes during this process.

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Poster 11

CIRM-CF, a French Biological Resource Centre dedicated to filamentous fungi for lignocellulosic biomass valorization

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The CIRM-CF (Centre International de Ressources Microbiennes – Champignons Filamenteux) is a microbial Biological Resource Centre (BRC) dedicated to the preservation of filamentous fungi of interest for agro-industries. This young and growing collection organizes collecting operations in varying biotopes, including tropical regions. The majority of fungal strains isolated are naturally efficient to degrade lignocellulose and aromatic compounds. In 2018, the CIRM-CF collection gathers over 500 species of filamentous fungi and over 2,000 lignocellulolytic strains (60% belonging to Basidiomycota and 40% to Ascomycota).

The CIRM-CF, in conjunction with the BBF lab (Marseille, France), benefits from an exceptional scientific environment that allows the identification of strains using classical taxonomy, molecular tools and enzymatic characterization. Aiming high quality standards, this BRC is ISO9001 certified since 2006 for acquisition, authentication, conservation and diffusion of its fungal resources. Exclusive strains and facilities are available (https://www6.inra.fr/cirm_eng/Filamentous-Fungi) to academic and industrial partners to explore the enzymatic potential of fungal biodiversity by using a high-throughput screening platform. These enzymes can be used in various white biotechnology processes for lignocellulosic biomass valorization.

The CIRM-CF belongs to the CIRM network, created by the INRA in 2004, to preserve and valorize microbial biodiversity. This network, identify as a BRC by IBiSA, makes available more than 15 000 strains, spread on 5 sites :

- *filamentous fungi of agro-industrial interest (Marseille, France)*
- *yeast of biotechnological interest (Grignon, France)*
- *bacteria of agro-alimentary interest (Rennes, France)*
- *pathogen bacteria of the food chain (Tours, France).*
- *phytopathogen bacteria (Angers, France)*

Poster 12

Ligninolytic potential of *Thermobacillus xylanilyticus* for the production of aromatic molecules

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Currently, lignin has a strong impact on the recalcitrance of plant biomass. Lignin is still underused, and mainly burned to produce heat, whereas many aromatic molecules can be extracted and used by industries [1]. The combination of chemical and biological processes such as ligninolytic enzymes will allow a better use of these biomolecules in biorefineries [1]. Many studies have been conducted on fungi for the identification of these ligninolytic enzymes but recently, the ability of various bacteria to break down lignin has been reported [2]. In this context, the present study aims to identify ligninolytic enzymes for the deconstruction of plant biomass, from a model bacterium known for its hemicellulolytic activities and its thermo-stability: *Thermobacillus xylanilyticus* [3, 4]. Indeed, oxidoreductase genes have been identified in the genome of the bacterium and expression of some of them measured on wheat straw substrate culture. The goal of this study is to investigate if *T. xylanilyticus* grown on various lignin-rich substrates (wheat straw, steam-exploded wheat straw and kraft lignin) is able to produce ligninolytic activities. The bacterial growth kinetic parameters were determined in relation with the production of phenol-oxidases and peroxidases activities required for the deconstruction of lignin in the three substrates. The ability of the bacterium to use and produce phenolic compounds (vanillin, ferulic acid ...) in the culture medium was also evaluated. Finally, the characterizations of the substrates before and after the growth of bacteria by Fourier Transform InfraRed spectroscopy (FTIR) demonstrate the effect of lignocellulolytic enzyme activities produced by *T. xylanilyticus*.

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Poster 13

Cellulose-degrading fungi from central Morocco: Comparative analysis of enzymatic activity, *in silico* prediction of functional properties and molecular docking

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The diversity of enzymes involved in cellulose degradation by fungi is a clear demonstration of the adaptability of these microorganisms to different biotopes and environmental changes. The secret behind their performance is particularly due to the production of different classes of cellulases, acting in synergy and complementarity. The most studied classes are Endoglucanases (EC 3.2.14), Exoglucanases (EC 3.2.1.91), β-glucosidases (EC 3.2.1.21) and the recently discovered, Lytic Polysaccharide Monooxygenase (LPMOs).

Structure - function relationship and prediction concept have promised benefits in the study of enzymes with the arrival of *in silico* analysis. The development of computational tools contributed to the discovery of pertinent and stable enzymes. Nowadays, the application of bio-informatics revolutionized the field of molecular biology and is key to a better characterization of functional properties of cellulolytic enzymes before their production at industrial scale. This comprehension is very crucial to biochemists and physiologists assisting them in the design of process operations from extraction, purification, separation to industrial applications of cellulolytic enzymes and cocktails.

This poster abstract represents a quantitative study of cellulolytic potential of two fungi strains isolated from decaying wood in central Morocco. A comparison is made between the measured cellulolytic activities and *in silico* prediction of physico-chemical and structure conformational properties. *Penicillium brasilianum* isolate was shown to be a potential cellulolytic fungus with 1.1 IU/ml total cellulase activity, 3.2 IU/ml endoglucanase activity and Xylanase activity of 41.7 IU/ml. Cellulose hydrolysis yield achieved 63.6%. However, *Trichoderma atroviride* presented less cellulose-degrading capacity and hydrolyzed the polysaccharide only to 1.26%. These results were concordant with computational study analysis, particularly for *P. brasilianum,* which produced very stable cellulases capable of strongly binding to the substrate. *T. atroviride* results showed evidence of proteomic diversity among isolates of the same species, conditioned by variation of environmental parameters.

Poster 14

Lignin decomposition for enhanced bioethanol production: Improving the process using a bio-pre-treatment with the fungus isolate *Humicola grisea* from central Morocco

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The decomposition of lignocellulosic biomass is a natural phenomenon performed by a diversity of bacteria and fungi in natural habitat. The role of this microbiome is very important in maintaining the carbon cycle and renewing the soil composition. On the other hand, the structural backbone of lignocellulose feedstocks is far of being that simple, a complexity that confers recalcitrance to the material and renders its bio-decomposition difficult. The major limits are the cellulose crystallinity, the composition in lignin polymer, the xylan structure and the formation of xylan-lignin complexes. Hence, a knowledge-based strategy should be adopted to achieve high sugar conversion rates and enable sustainable second generation bioethanol production. Pretreatment of lignocellulosic biomass is a first solution assuring accessibility of the substrate to the cellulolytic enzymes. The process should be sustainable avoiding large amounts of chemicals and without the formation of fermentation inhibitors. Pretreatment strategies using fungi and microbial consortia are thus privileged.

This study provides a first report on the potential use of a fungus isolate, *Humicola grisea* from central Morocco, as a high performance lignin decomposer to construct a sustainable biomass pretreatment bioprocess. The strain was isolated from decaying wood, identified with molecular technique and characterized for the kinetics of production of lignolytic enzymes in liquid culture. This wild-type fungus was shown to be relevant producer of ligninases with 8.6 IU/ml - 11.3IU/ml and 1.1 IU/ml, laccase, lignin peroxidase and manganese peroxidase activities, respectively. Maximum activities were measured on the fifth day of incubation showing a unique optimum point for the three classes of ligninases, reflecting the high synergy that exists between the lignin-degrading enzymes. For an industrial process, use of an enzymatic extract of the fungus is suggested rather than direct inoculation in order to enhance process efficiency, avoid long treatment times and possible sugar consumption by the fungus.

Poster 15

Deciphering the molecular mechanisms of wood extractives toxicity using *Phanerochaete Chysosporium* mutants

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Phanerochaete chrysosporium is a white rot fungus able to degrade all components of wood. The efficiency of degradation is not only based on the extracellular enzymatic systems to decompose both cellulose and lignin but also to the ability of the fungus to cope with various compounds that are released during the process. Among these molecules, extractives that are non-structural components of wood, are toxic to fungi leading to wood durability. To investigate the response of *P. chrysosporium* to the toxicity of wood extractives without *a priori*, we performed a forward genetic strategy using mutagenesis and screening to identify genes involved in the resistance/detoxification process developed by the fungus. Before to start screening with wood extractives that are mixes of molecules, a proof of concept of this strategy has been provided using singles molecules with known antifungal activities. These experiments led to the identification of the molecular target of these compounds in *P. chrysosporium*. Altogether these results and the strategy can provide tools for using some recalcitrant lignocellulosic materials.

Poster 16

What form of fungal inoculum for degradation of miscanthus under non-sterile solid state cultivation, on pre-pilot fermentor?

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Faced with increasing food and energy needs, plant biomass is generating new interests. A new alternative to the deconstruction of this raw materials is biological pre-treatment using wood-decay fungi. Indeed, the elimination of lignin increases the accessibility of cellulose and hemicellulose to hydrolytic enzymes for sugar release.

In laboratory, fungal pretreatment is usually performed via sterilized solid-state cultivation, but the difficulties are the non-sterile conditions of culture in scale-up at the pilot and industrial levels. Indeed, fungi can be sensitive to competition with indigenous microorganisms present on the feedstock and to variations in environmental moisture present during growth [1].

Therefore we tested a white rot decay fungus, *Leiotrametes menziesii* (BRFM 1368) on its ability to grow on miscanthus straw, a perennial grass, under non-sterile culture conditions, on pre-pilot solid state fermentation SSF (Fujiwara fermentor). Two types of inocula are prepared under sterile conditions: a liquid culture inoculum in Roux flask (7 x 200 mL MA_2 medium) and a solid culture inoculum on miscanthus granules (700 g DM, moisture content 75%). After 10 days of static incubation at 33°C, these two inocula are delicately fractionated and mixed with 1kg (DM) of autoclaved or non-autoclaved miscanthus straw (moisture content 68 %). These 4 inoculated culture media are left 23 days at 33°C in non-sterile condition in the fermenter.

Visual and pH monitoring of the environment and material sampling are performed, approximately every 7 days, in order to estimate the growth of fungus and to analyze the composition of the residual straw. The selectivity degradation for lignin of the four tested conditions, based on ratio of lignin degradation over cellulose consumption is measured.

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Poster 17

Straw removal affects N_2O emission in sugarcane cropping systems

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After sugarcane green harvest in Brazil, high amount of lignocellulosic biomass is left at the soil surface and potentially exported for biofuel production. Recycling of crop residues and the presence of mulch in such cropping systems, influence the fate of fertilizer-N, the storage of C in soil, but also the potential emissions of greenhouse gas (N_2O). In this study we examine how the quantity of crop residues at the soil surface and the fertilization, influence the fate of residue-C and the N_2O emission in sugarcane cropping system. The study was carried out in field, at the University Federal of Santa Maria (southern Brazil) under subtropical climatic conditions. Four amounts of crop residues, i.e., 0, 4, 8 and 12 Mg DM ha^{-1} (equivalent to 100, 67, 33 and 0% straw removal in this area) were combined with fertilization (0 and 100 kg urea-N ha^{-1}), which was applied in a single dose at day 52 after sugarcane harvest. We quantified during one year after sugarcane harvest, remaining residue-C using destructive microplots once a month, and N_2O fluxes two to three times per week during the first two months and less frequently thereafter, using static chamber. Our results showed that the C loss was proportional to the initial amount of residues and was not affected by N fertilization. N_2O emissions were favoured by higher amount of straw (12S > 8S > 4S > 0S) with and without fertilizer-N. Removal of straw decreased C available for microorganisms and decreased mulch moisture and soil water field pore space (WFPS) by increasing water evaporation, both factors responsible for the production of N_2O in soil. These results indicates that the climate mitigation potential of soil C management could be overestimated by neglecting N_2O emissions, and that management of lignocellulosic biomasses at crop harvest strongly impact both processes.

Poster 18

Gaseous Emissions of Nitrogen Species from Decomposing Crop Residues: Construction and Calibration of a Novel Model

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Synergy between agriculture and environment is crucial for sustaining a healthy livelihood. The unharvested plant biomass in agricultural fields is a rich nutrient resource for soils and constitutes a potential for carbon sequestration. Microbial action and consequent breaking down of organic matter from residues enhance soil microbial activity and increase organic matter content. However, organic matter decomposition also releases carbon dioxide (CO_2) and nitrous oxide (N_2O), as well as other compounds to the atmosphere. These emissions contribute to additional radiative forcing and air pollution. In order to quantify potential benefits and drawbacks of residue incorporation practices, it is therefore essential to characterise gaseous emissions from crop residues. This requires understanding how crop residues quality influences their microbial breakdown in soils. Current gas emission models account for meteorology, soil characteristics, carbon and nitrogen contents, and fertiliser application. These models however do not account for the effects of organic matter quality on the decomposition rates and on greenhouse gas (GHG) emissions while in organic matter decomposition models, they explicitly account for heterotrophic microbial activity and for the effect of residue quality on soil nitrogen fluxes and carbon sequestration. Our objective is to utilise available literature, expert knowledge, results and observations from dedicated laboratory experiments, to develop and calibrate a new model coupling both gaseous emissions to the atmosphere, organic matter decomposition in the soil and accounting for agricultural practice through its effect on the quality of crop residues. We will parametrise the model by an optimisation process by using results of experimental incubations of residues, in the prototype Gas Emission Detection Incubator (GEDI), for a large range of residues quality, management options and soil conditions.

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Poster 19

Toward production of furfurylic alcohol using green chemistry and white technology

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Beech is a very abundant resource in France but the low natural durability and dimensional stability of the beech wood dramatically limit its use in building industry. Beech wood performances can be greatly improved by timber impregnation with furfurylic alcohol (FA) and subsequent *in situ* polymerization. Such wood composites are currently designed for high-end market. The work presented here explores the potential of a 3 steps batch process to perform a sustainable conversion of beech hemicelluloses into bio-based furfurylic alcohol (FA) which can be used latter on for beech wood treatment. The first step consists in the enzymatic conversion of xylans, the main components of the beech wood hemicellulose, to xylose by a cooperative action of xylanases and β-xylosidases enzymes obtained from submerged fermentation of *Trichoderma reesei*. Biochemical and microscopic characterization showed a synergic action of lactose and beech sawdust on xylanases and β-xylosidases production mainly found in the sawdust associated hyphae. Furfural is a poisonous compound. Thus, dehydration of xylose to furfural was achieved by using mild acid catalyzed chemical conversion instead of microorganisms. Using acetic acid and beech coal as heterogeneous catalyst, furfural was produced with a 48% yield in 5h. We used *Saccharomyces cerevisiae* ability to convert furfural to FA with high conversion rate as a biotechnological alternative to the industrial conversion using copper chromite used as catalyst. Furfural conversion rate and FA yield were 86% and 57% respectively. Interestingly, *S. cerevisiae* growth was not inhibited by furfural and acetic acid occurring in medium, avoiding purification between steps 2 and 3. We showed here that beech sawdust could be used as source biomass to produce bio-based FA. We are currently investigating the use of instrumented bioreactors to perform the steps 1 and 3 and integrate them in a single continuous process.

Poster 20

Chemo-enzymatic synthesis of renewable antioxidant additives with tunable polarity from lignocellulose and vegetal oil

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Antioxidant activity is one of the major properties needed in packaging. Antioxidants protect goods and also packaging itself from aging. This ability is given by additives present in polymer matrices, and they obey to strict regulations (e.g., REACH). Unfortunately, these molecules are often petro-based and may occasionally be toxic (e.g., BHT). Moreover, the choice of the antioxidant structure may be limited by factors such as volatility and solubility.

Today, the development of biorefineries opens new perspectives for the development of potent and eco-friendly antioxidant additives. Lignocellulosic biomass, which is rich in hindered phenolics acting like free radicals scavengers, can provide structures with potent antioxidant properties. On the other hand, oleaginous biomass is an abundant source of renewable apolar aliphatic chains.

In this work, we have developed a novel family of renewable phenolic compounds from lignocellulose (e.g., ferulic acid) and oleaginous biomasses (e.g., fatty acids) using a chemo-enzymatic process. In order to avoid volatility issues and warrant an efficient solubility during their incorporation in polyolefins, their polarities have been judiciously designed by playing with their chemical structure (SAR).

The antiradical activities and CMC of these bisphenols were then determined.

Poster 21

One pot process for the production of diformylfuran and its transformation into gemini surfactants with particularly low critical micelle concentration

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Biomass or biomass derived compounds are ideal starting material for the synthesis of particularly functionalized platform chemicals. Thus furans such as hydroxymethylfurfural (HMR) or diformylfuran (DFF) are obtained from hexose containing biomass. Although possessing two reactive aldehyde functions, DFF was less frequently considered as a suitable intermediate in fine chemistry. Most frequently it is obtained from HMF. We have developed a one-pot procedure for the synthesis of DFF starting form hexoses [1]. The process can also be applied to the corresponding transformation of primary biomass such as wheat bran or starch. DFF obtained from this procedure was transformed into gemini surfactants, most of them possessing a very low critical micellar concentration (1.5 µmol L^{-1}) [2]. In gemini surfactants two detergent moieties are connected [3]. In the case of our compounds, a particular relationship between the surface excess *Γ* and the bulk concentration is observed. *Γ* does not necessarily correspond to a saturation of the surface with the surfactant. These new surfactants also possess bactericidal and fungicidal properties.

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Poster 22

ValBran: valorization of wheat bran into green surfactants

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The ValBran project, started in January 2017, develops original and environmentally friendly biotechnological and green chemistry pathways for the production of various surfactant molecules from wheat bran. Molecules with high added value for various applications (cosmetics, detergents, phytosanitary agents…) are targeted. The approach is dedicated to the development of several laboratory-scale transformation pathways to fractionate polysaccharides from wheat bran and to enzymatically functionalize the sugars obtained into alkyl glycosides -or into sugar esters. Structures and physico-chemical properties of generated surfactant molecules are investigated. The most promisingpathway(s) will be selected for pilot up-scaling in order to obtain economic and environmental impact of the developed process(es). Wheat bran residues generated during the process will be of interest for animal feed.

This project involves the University of Reims Champagne-Ardenne, the University of Picardie Jules Verne, the French competitiveness cluster “Industries des Agro-Ressources” (IAR). In Wallonia the University of Liège, the Walloon association ValBiom and the Greenwin cluster. In Flanders, the project includes the VITO research and technology center, the INAGRO association and the Catalisti cluster.

Poster 23

Wheat Bran pretreatment by Room Temperature Ionic Liquid-water mixture: Optimization of operating conditions by screening design

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Wheat bran (WB) is one of the most important agricultural co-product produced by milling and biorefineries. WB is an abundant source of hemicellulose (38-55%) and cellulose (16-30%) and constitutes an attractive low cost biomass for the production of platform molecules or fermentable sugars [1]. However, the recalcitrant structure of this biomass constrains the enzymatic conversion (of the carbohydrate fraction) into sugar mono/oligomers requiring thus a preliminary pretreatment step. Pretreatment of biomass with room temperature ionic liquids (RTILs) have attracted attention in the last years due to their capacity to disrupt the rigid structure of biomass, recyclability, high thermal stability as well as to their ecofriendliness for some of them [2,3]. This study was conducted to evaluate the effect of pretreatment of Wheat Bran (WB) and Destarched Wheat Bran (DWB) with RTILs, to improve the enzymatic hydrolysis of cellulose and xylans. In order to get an efficient pretreatment and to maximize sugar yields from the enzymatic hydrolysis, the influence of main operating conditions such as pretreatment temperature, time, solid loading (RTIL to WB or DWB ratio) and RTIL percentage (RTIL to water ratio) were investigated and optimized through a factorial screening design using Statgraphics Centurion XVIII® software. Experimental design included 2 blocks and two levels of the 5 independent variables for a total of 36 experiments. This experimental design allowed estimating the main effects and interactions amongst all factors. Thus, a multiple linear regression model describing the sugar yields as a function of significant pretreatment factors was established. This study was supplemented by a chemical composition analysis and structural characterization of untreated and pretreated WB and DWB biomasses. The results presented in this study form part of European Interreg ValBran project (www.valbran.eu).

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Poster 24

From Bench Scale to kilolab Production of Renewable Ferulic Acid-based Bisphenols: Optimisation and Evaluation of Different Purification Approaches Towards a Technical Feasibility and Process Environmental Sustainability

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This work reports on the assessment and optimisation of different separation and purification approaches for renewable macrobisphenols[1] (***IDF*** *(bis-O-dihydroferuloylisosorbide),* ***BDF*** *(bis-O-dihydroferuloyl 1,4-butanediol),* obtained in our laboratory from bio-based polyols and ferulic acid in a previous work[2]. **BDF** was successfully synthesized and purified by recristallization at kilolab scale with a purity of 95%. However, recristallization was unsuccessful for other macrobisphenols. Therefore, solvent resistant nanofiltration (SRNF) was tested as an alternative purification method that offers the possibility of separating the species on the basis of their different molecular weight.

In this context, six solvent resistant membranes with cut-off between 200 Da and 500 Da were evaluated for the separation of a mixture containing 1 g/L of **BDF/EtDFe** (*ethyl dihydroferulate*) 80/20 w/w% in acetone, using a cross-flow separation system. The membrane presenting the best separation performance (permeate flux and rejection) was used for a further study at the optimal transmembrane pressure previously determined through membrane screening. At this stage, constant-volume diananofiltration was set-up in a single and in a two stages process (cascade concept) in order to assess the improvement in product yield recovery. Results showed that a two stage diafiltration reduces product losses from 23% to 5%. Besides, **BDF** was successfully separated from **EtDFe**, with a 95% purity. Furthermore, the solvent used was recovered and regenerated by an additional stage of nanofiltration, which allowed the recovery of 90% of the initial solvent with less than 1% of impurities.

A Life Cycle Analysis (LCA) was performed and compared to the one related to purification of **BDF** using recrystallization. Results showed that only the integration of solvent recycling in the membrane separation process, using a starting solution with a total macrobisphenol concentration of 150 g/L, lead to a similar environmental footprint compared to the one found for recrystallization.

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Poster 25

Effect of alkaline pre-treatment of giant reed biomass on biogas production

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Lignocellulosic materials can be converted into bioethanol, bio-oil or biomethane, and pre-treatments are recommended to enhance most of these processes, removing lignin and exposing holocellulose. Giant reed (*Arundo donax*) is a perennial lignocellulosic grass, currently regarded as an emerging bioenergy crop, with high biomass yield and low input requirement. Within the framework of the AGROENER project funded by MiPAAF, Italy (D.D. 26329/2016), studies are ongoing on the suitability of giant reed biomass as substrate for second generation biofuels, as well as on the prototyping of the production of high quality inocula for anaerobic digestion. The aim of this study was to evaluate the effect of a pre-treatment of giant reed biomass with diluted alkali (10% w/w slurry of oven-dried biomass, KOH 1.5% w/w, 121 °C, 20 min) on methane production by anaerobic digestion, in laboratory static batch conditions (100 ml reactors, 1 g of pre-treated dry biomass, 35°C). The impact of possible inhibitors released by the pre-treatment was evaluated by comparing the biogas production from unwashed and washed materials. In addition, to assess the pre-treatment effectiveness, samples of the pre-treated materials were enzymatically hydrolysed (25 FPU/g) and the sugar release was followed up to 144 h. The final sugar yield increased by 60%, from 150 mg/g in the control (0% KOH) up to 418 mg/g in the alkali pre-treated samples (360 mg/g, if washed). These results indicate that the biomass was effectively delignified. Considering biogas production, the alkali pre-treatment without washing had no inhibitory effect, as no differences in lag phase were observed between treatments. Thus, after KOH pre-treatment, the detoxifying washing step is not necessary. The cumulative methane production, calculated at 45 d by a modified Gompertz equation, was 24% higher for unwashed KOH pre-treated samples than for the control (KOH 0%).

Poster 26

New sugar-based amphiphilic compounds with surfactant or gelling properties – focus on xylose from ligno-cellulosic biomass

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In last few decades, Green Chemistry concept has emerged as a solution to reduce environmental impact of chemical compounds and processes. So bio-based compounds were widely studied due to the increasing interest for development of eco-friendly and biocompatible products from renewable sources.

Sugar-based compounds have been developed in the field of gelators and surfactants to try to replace petrochemically-based equivalents in several domains. Initially developed around agro-resources, bio-based chemistry is now turning towards the use of ligno-cellulosic biomass from wood. More precisely in our case, by-products of wood industry may be valorised by using cellulosic and hemi-cellulosic parts of wood as renewable sources of sugars.

This study focused on the main pentose from hemi-cellulosic part of beech wood: the xylose. Beech is predominantly present in Lorraine and border regions and countries but is relatively little valorised.

The aim of this work is to chemically modified the xylose issued from lingo-cellulosic biomass to design new amphiphilic compounds with surfactant or gelling properties for cosmetics or pharmaceutics.

Synthesis are designed to respect as closely as possible Green Chemistry principles. Properties of each compound are characterized.

Poster 27

Xylitol production from lignocellulosic hydrolysates through fermentation after detoxification by nanofiltration

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Lignocellulosic biomass (LCB) is highly studied as a source of renewable energy, particularly through the exploitation of cellulose as a substrate for the production of bioethanol. Accessing the cellulose from LCB requires a cracking method. Steam explosion in acidic conditions is one of the most efficient techniques. The by-product is a liquid hydrolysate containing fermentable sugars (mainly xylose and glucose). However, the drastic conditions applied for the extraction of cellulose, e.g. low pH and high sulfate concentration, make it an unfriendly environment for microorganisms to develop resulting in low fermentation activity. Furthermore, the biomass pretreatment releases carboxylic and phenolic acids as well as molecules derived from sugar breakdown (furfural and Hydroxymethylfurfural (HMF)), which display strong antimicrobial activities even at low concentrations.

The strategy employed here to increase the fermentability of LCB hydrolysates, was to separate the sugars from the inhibitory molecules by membrane diananofiltration. This filtration step applied on wheat straw hydrolysates, was able to efficiently remove HMF and furfural, carboxylic acids (e.g. acetic and formic acids) as well as significant proportion of the phenolic acids. To evaluate the fermentability of the detoxified hydrolysate, three different bacteria (*Bacillus subtilis* 168, *Lactobacillus reuteri* DSM 17938 and *Pseudomonas fluorescens* DSM8369) and four yeasts (*Debaryomyces hansenii* CLIB607, *Kluyveromyces marxianus* CLIB1533, *Scheffersomyces stipitis* CLIB187, *Meyerozyma guilliermondii* CLIB222 and *Brettanomyces naardenensis* CLIB1178) were cultivated on hydrolysates as a growth medium after pH adjustment. Raw and detoxified hydrolysates appeared unsuitable for the cultivation of bacteria whereas all yeasts were able to grow on detoxified medium. However, no yeast growth could be observed on raw medium. Furthermore, three yeasts strains (CLIB222, CLIB607 and CLIB1178) allowed a good conversion of xylose to xylitol. In particular, *B. naardenensis* exhibited a better conversion yield, up to 0.51 gxylitol/gxylose than the better-known *D. Hansenii* 0.40 $g_{xylitol}/g_{xylose}$ and *M. Guilliermondi* 0.45 $g_{xylitol}/g_{xylose}$.

Poster 28

Antimicrobial activity assessment of phenolic compounds derived from vanillin using flow cytometry

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Public concern about additives in food products and cosmetics leads to a raise in the demand for natural harmless preservatives. To answer this issue, natural phenolics chemistry offers a range of possibilities to develop biobased polymers. In particular, oligomers of natural phenolic molecules are interesting alternatives to bisphenol A [1]. In addition to their interest as polymer building blocks, antimicrobial properties of phenolic compounds are other advantageous characteristics for their incorporation into plastic formulations. The wide spectrum of structural features of natural phenolic molecules raises the question of the structure-function relationship linked to antimicrobial activity.

In order to assess the role of chemical functions of natural phenolic compounds in their antimicrobial activity, different molecules based on vanillin were synthetised. Bis-vanillin, acetylated vanillin and acetylated bis-vanillin were compared to eugenol and ferulic acid for their antimicrobial activities on *E.coli* and *B.subtilis* by monitoring growth curves in liquid medium, using 96-well microplates reader. Additionally, flow cytometry analysis were carried out to elucidate the mode of action of the tested molecules on the bacteria's physiological features, i.e, membrane integrity by propidium iodide (PI) accumulation, esterases activity by Chemchrom V8 clivage, membrane potential by DiBaC accumulation and membrane fluidity by membrane fluorescence anisotropy [2].

The antimicrobial activity of the different molecules was first assessed by monitoring bacterial growth parameters, i.e lag time and growth rate, as a function of molecules concentration. Dimers and acetylated molecules impacted more strongly the growth parameters, whatever the considered microorganism. However, the cellular responses depended both on the bacteria species and on the molecule; as acetylated vanillin caused major cell depolarisation, dimerisation impacted membrane fluidity. Flow cytometry, combined to growth curves analysis, appeared to be a good method to understand how phenolic compounds interact with bacteria and help formulating antimicrobial products.

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Poster 29

LIGNDEVI project: Lignin as an antioxidant ingredient

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LIGNDEVI (LIGNine as source of functional ingredient development) is a project supported by the Fédération Jacques Villermaux, a six-laboratory federation covering the fields of energy, mechanics, processes and product engineering. This project uses a multidisciplinary approach and its goal is to develop new products containing industrial lignin as an antioxidant ingredient.

Lignin one of the three major polymeric constituents of the lignocellulosic biomass, representing 20 to 40 % of the mass of most wood species [1]. Industries produce each year about 50 million tons of industrial lignin, and the main part comes from the pulp and paper industry [2]. The largest part of this lignin is burned for energy, and only a small fraction is currently used for chemical or material applications. Second-generation biorefineries development also increases the need to find industrial uses of lignin to create a complete business model. Two options are generally considered for lignin valorization; the first is to depolymerize lignin to obtain high-value added phenols [3]. Here, the second valorization way is preferred [4]: we use polymeric or oligomeric lignins without major chemical modification as ingredients of formulated products.

Chemical Product Design, a method of formalizing innovation for new chemical products, is used to help us to highlight potential scientific and industrial locks. To maximize the chances of success with this method, communication with industrial partners is a key point and their input will be very helpful to set up specifications for the targeted products.

The first study of this project is a micronization of the lignin. Organosolv lignin is produced from beech wood is ball-milled and sonicated. Grinded lignin modifications are estimated by analytical methods such as antioxidant activity tests, size exclusion chromatography and NMR. The next step for this project is the production and characterization of composites material mixed with lignin particles.

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Poster 30

Thermodynamics and Kinetics Screening of Cellulose Fiber Dissolution in Ionic Liquids

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There has been an insistent demand for 'green' materials in the last decades leading to the development of new all-polymer composites, all-cellulose composites (ACCs) [1-5]. The latter are composed of a cellulose matrix reinforced with partially dissolved cellulose fibers. The evolution of fibers' behaviour in cellulose solvents is thus a very important step to be studied and understood to control ACC properties. In terms of building the strategy for making strong ACCs, it is of importance to guarantee partial dissolution rather than dismantling or complete dissolution of cellulose fiber.

The aim of this study is to understand the dissolution kinetics of fibers from different sources, man-made and natural, in two ionic liquids (ILs). To do this, the evolution of fibers dissolution was monitored by Optical Microscope at various temperatures, and correlated with the dissolution enthalpy calculated by using DSC. Furthermore, the basic chemical and physical properties of both fibers and ILs were carefully characterised, including chemical composition, molecular mass distribution, crystallinity and morphology of cellulose fibers together with moisture contents, thermal and rheological properties of ILs. The fiber structure-dissolution relationship is discussed based on the results obtained, attempting to propose controlled designs of ACCs.

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Poster 31

Which recycling routes for biocomposites?

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The impetus of governmental or industrial directives and the need to reduce the environmental footprint of materials requires a global reflection on their end-of-life, including for biocomposites. For about a decade, plant fibres composites have demonstrated their potential of recyclability, particularly thanks to the good behaviour of plant reinforcements after processing [1]. This work presents a synthesis of various works centred on the recycling of thermoplastic biocomposites based on flax fibres, but also wood, by extrusion and injection.

Among a wide range of results, we will mention the changes in fibre lengths during the process, which generally decrease quite sharply after a first processing cycle [2]. Our results show that it can nevertheless be moderate depending on the recycling method chosen. Interestingly, this decrease can be related to the viscosity of the matrix but also to the structural defects initially present in the plant fibres [3]. We will also highlight the role of fine particles, generated by fibre preparation or the process, in the performance of materials. The results presented also highlight the impact of a thermal cycle on mechanical performance at the fibre and plant cell wall level. A link can be established with biochemical changes in the plant walls, which differ according to the nature of the fibre [4].

In addition to this conventional recycling, new approaches are proposed by thermocompression and also by reincorporation in non-woven materials [5].

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Poster 32

Thermoplastic/lignocellulosic fibres composites: from preparation to properties

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Lignocellulosic fibers (flax, hemp, sisal…) are more and more considered as reinforcing fibers to prepare thermoplastic-based composites. Compared to classical glass or carbon fibers, they are abundant, renewable, cheaper, and allow to reduce parts weight, what is an important issue for automotive applications. However, during compounding with a thermoplastic matrix, fiber bundles suffer important degradation, resulting in a severe reduction of length, diameter and aspect ratio. As the mechanical properties of the final parts are depending on fiber dimensions, it is important to understand why and how lignocellulosic fibers break during a compounding process.

In this work, we performed experiments with various lignocellulosic fibers and a polypropylene matrix. The composites were prepared using a twin-screw extruder under different processing conditions. Samples were collected at the die exit and along the screws. The dimensions of the fibers were analysed by image analysis after matrix dissolution. It was shown that the fibers length and aspect ratio are reduced as soon as the fibers are introduced and mixed with the molten matrix. Both dimensions follow a regular decrease along the screw profile, which can be described by exponential functions, according to the strain experienced by the fibers. By coupling these evolution laws with a flow model of the extrusion process, it is possible to elaborate a predictive model of the fibers breakage, which can then be used to optimize the process.

Poster 33

Evaluation of the lignocellulose hydrolysate materials as a substrate for the sustainable production of high-value single cell oils

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Oleaginous fungi (filamentous fungi and yeasts), grown under nitrogen limiting growth conditions, are able to accumulate lipids – single cell oils, in high amounts. The accumulated lipids are in the form of triacylglicerides (TAGs) and their fatty acid profiles are in many cases similar to the fatty acid profile of plant or fish oils [1]. In addition, oleaginous fungi can utilize a variety of materials as substrates. Even lignocellulose hydrolysate materials can be used as substrates for the single cell oil production [2]. Due to these reasons, oleaginous fungi are considered as an alternative source to high-value oils for use as animal nutrition and food additives for humans. The composition of substrates based on lignocellulose hydrolysates can be very complex and vary depending on the source of lignocellulose. Some of the components may have positive effects on growth and other components are inhibitors for lipid accumulation in fungal cells. Thus, there is a need for screening of high numbers of oleaginous fungi on different types of lignocellulose hydrolysate substrates, in order to optimise lipid production processes from the lignocellulosic hydrolysates and in order to identify growth inhibitors. Recently, our group developed a screening setup based on the high-throughput Duetz micro-cultivation system. The new screening setup combines the Duetz system with Fourier-transform infrared spectroscopy (FTIR) and automated sample handling for rapid and non-destructive screening of oleaginous fungi [3].

We used the screening setup for screening different types of lignocellulose hydrolysate materials and different types of oleaginous fungi. The oleaginous fungi employed are good producers of high- and low-value lipids. The analysis of fungal biomass was performed by FTIR spectroscopy and the analysis of lipids was performed by GC-FID chromatography.

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Poster 34

Biobased polyesters for non-biocidal beech protection

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Wood is susceptible to dimensional instability and low durability due to water sorption processes occurring during its service life, especially for outdoor applications. Chemical modification methods have been extensively studied during past decades [1]. Beyond them, acetylation and furfurylation processes succeeded in industrial implementations, Accoya® and Kebony®. In this context, our laboratories are continuously involved in the development of wood chemical modification.

The two-step process consists first in a vacuum /pressure impregnation of wood with polyesters precursors, then impregnated wood is oven-cured in order to induce *in situ* polymerization in the wood cell wall and fix the thermoset polymer. The choice of beech (*Fagus sylvatica*) as wood substrate is particularly pertinent, as this species is particularly water sensible, non durable and very abundant in Europe. Significant improvement of its properties could promote new valorizations notably in construction area. Our approach aims to develop biobased and non ecotoxic chemical treatments starting from key molecules originated from biomass deconstruction [2,3]:
- lactic acid (LA) or oligomers (LAO) [5]
- glycerol and polyhydroxylic acids such as tartaric and citric acids [4] and one derivate from succinic acid.

Varying treatment conditions have been studied in order to select optimal treatment for each route, avoiding product leaching off and mechanical weakening. The presentation brings to enlighten how treated beech properties such as dimensional stability and resistance to decay have been successfully improved by this method. Some perspectives of development are given.

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Poster 35

Influence of biomass dry fractionation on the processability and quality of 3D printable biomaterials from rice husk

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The introduction of biomass produced as side streams of primary industries in plastics/bioplastics has shown potential in reducing the used of polymers and in transferring some advantageous properties of the biomass to the final products (e.g. UV stability, stiffness). However, one particular challenge is related to the processing of the biomass prior to its incorporation in bioplastics as it can hinder the process or lead to poor quality parts [1]. These limitations are linked to the biomass water content, particle size, aspect ratio, dispersion and compatibility, and are particularly critical in the development of tailored biomass additives for 3D printing feedstocks. Dry fractionation processes by combining milling and sorting step have shown their potential to prepare tailor made powder that can used for many application (energy, materials green-chemistry…) [2]. Rice husk, composed of roughly 85 % lignocellulosic material and 15 % amorphous silica, is an agricultural by-product of huge tonnage weakly valuated because of its high mineral content. In this work we investigate the potential of rice husk powder as a filler for biomaterials intended to additive manufacturing (FDM) by studying the influence on the particle size distribution and the chemical composition of different fractions from rice husk on the processability and the quality of the filaments and 3D printed exemplars.

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Poster 36

Design of novel hydrophobic tannin foams

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Nowadays a lot of research studies deal with the design, the characterization and the development of new biosourced materials as opposed to synthetic products from non-renewable fossil fuels. Tannin-furanic foams are obtained by a mixed polymerization of furfuryl alcohol and tannins and are therefore made up of up to 90% of biosourced compounds. For this reason they are considered as a trustful alternative to phenolic or polyurethane foams for very diverse applications. Thanks to their low cost, their high resistance to compression, their self-extinguishing and insulating properties, this new generation of foam is highly considered for building insulation. A second objective targets the use of these same polymer for wood preservation in order to avoid the utilization of biocides.

The described work aims to design copolymers based on furfuryl alcohol and condensed tannins, or even flavonoid monomers such as catechin, modified by grafting one or more fatty acid chains to obtain hydrophobic materials. Indeed traditional tannin foams usually suffer from sensitivity to water and humidity due to their hydrophilic character.

Our approach presents a hydrophobization step of the tannin molecules, by way of grafting of natural fatty acids, and the subsequent foaming process. The hydrophobic character of the final construct can be modulated with the length of the fatty chain that is being used. This method has been first set up on model flavonoids before scale up to tannin modification. The foaming step has been performed using mixtures of natural tannins and hydrophobized tannins or natural tannins and hydrophobized flavonoids. A third option has been considered, consisting of the final hydrophobization of the pre-formed tannin foam.

This topic deals on the one hand mostly with applied research and should lead to the development of biosourced innovative materials. On the other hand fundamental aspects regarding tannin chemical reactivity are also discussed.

Poster 37

A novel biocarrier for immobilization of enzymes

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Our laboratory has developed a valorization approach of lignocellulosic biomass by fungal fermentation. The objective is to produce high-added value molecules such as enzymes, which are secreted during and for conversion from lignocellulose to fungal biomass.

Fungal enzymes are widely used in feed and food sectors. Notably, it has been shown that supplementation of fungal enzymes to animal diet improves the growth performance of monogastric animals [1], therefore the market of such enzymes is continuously growing [2]. However, the low shelf life of these enzymes forces the usage of immobilization techniques on solid substrate that have to be specific for each subset of enzymes [3]. In this context, our laboratory has elaborated a novel biocarrier for immobilization of enzymes mostly composed of a mixture of polysaccharides obtained after fungal fermentation of lignocellulosic biomass. Using this biocarrier, we have shown that the most valuable fungal enzymes can now be stored for prolonged period at room temperature, instead of only one month using common support. Moreover, we have shown that this biocarrier increases the thermal stability of the fungal enzymes showing no loss of activity after two days of heat treatment up to 60°C.

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Poster 38

Improvement of Wood Durability by industrial lignin impregnation

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The low durability of European wood species limits their use without biocide treatment. The traditional wood protection methods use chemicals that are considered toxic to the environment and can harm human health. To propose an ecological and environmental friendly alternative to biocides used in wood preservation treatment, lignin, one of the main wood components with a natural bio-protective property, was employed to impregnate beech and Scott pine wood samples. Different lignins, coming from different wood origins, were produced from black liquor, a by-product of chemical pulp industry, and more particularly from Kraft pulping black liquor. After the Kraft lignin production optimization, lignins were first selected for their antifungal effect on blue strain, white-rot and brown-rot fungi. Although all lignins showed a reduction in fungal growth, hardwood Kraft lignin totally inhibited *Coniophora puteana* growth. Softwood (Scott pine) and hardwood (beech) samples were impregnated with the different lignins preparations. Lignin concentration and impregnation protocols (pressure, vacuum and duration) were optimized to confer a good lignin distribution into the wood samples. After lignin impregnation, wood samples were lixiviated into water for one week to follow-up lignin release in this water. Despite of beech wood more impregnable and its higher lignin concentration, treatment of Scott pine wood samples was more effective after lixiviation. Wood weight losses, brought about fungal development, were significantly decreased if wood samples were treated with lignin. Then for Scott pine wood samples treated with hardwood lignin, a mass loss below 1% was measured (compared to the control ~ 23%), which proved a good efficiency of lignin treatment as wood protection to improve durability.

Poster 39

Extraction and valorisation of high molecular weight hemicelluloses from spruce wood

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Hemicelluloses are the second most abundant polysaccharide in nature after cellulose. Based on their functional and physicochemical properties, polymeric hemicelluloses have an important application potential in several fields such as pulp and paper making, packaging, and food industry. Hemicelluloses are present in lignocellulosic materials. However, the extraction of these compounds is highly limited by a network of lignocellulosic fibers. Therefore, lignocellulosic materials must be subjected to several treatments to improve deconstruction of the matrix and allowing easier release of hemicelluloses. The present study concerns the evaluation of the efficiency of steam explosion (STEX) on polymeric form of hemicelluloses extraction from spruce wood.

The effect of STEX on biomass combines chemical hydrolysis during the steam treatment and defibration during the explosive decompression. After impregnation in water (neutral medium) or 1M NaOH solution (basic medium), spruce sawdust (1-5 mm) was treated with hot steam (170°C, 190°C or 200°C) under pressure (around 1.5-3 MPa) during few minutes (2, 5, 10 or 30 min) followed by an explosive decompression. After each experiment, polymeric hemicelluloses were recovered by ethanol precipitation and dialysis.

For both media (neutral and basic conditions), the highest yields were obtained at 190°C during 10 mn (58 and 43 mg/g or dry wood respectively). For longer duration and/or higher STEX temperatures, lower polymeric hemicelluloses yields with lower molecular mass were obtained because of subsequent degradations. Interestingly, a high extraction selectivity was observed as a function of the pH of the impregnation step: relatively low molecular mass acetylated galactoglucomannans (~30 kDa) were isolated after a neutral impregnation and high molecular mass arabinoglucoronoxylans (~70 kDa) in basic conditions.

Films from extracted hemicelluloses were produced by casting the film-forming solutions, followed by solvent evaporation at a temperature of 60 °C. The physical properties such as thickness, solubility, oxygen permeability, water vapor transfer rate, surface structure and color of the hemicelluloses films were investigated, the study is still in progress.

Poster 40

The 3D_BioMat project: real and virtual exploration of lignocellulosic materials at a sub-micrometric scale

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The biosourced materials sector is expanding rapidly worldwide, driven by market demand for renewable products, incentives and regulatory constraints. With 80% of Europe's annual fibre production and a forest still underexploited, France should benefit from these market prospects, provided it is able to produce high-performance materials and meet user demand.

The 3D_BioMat project takes advantage of the latest advances in materials science: it combines sub-micrometric 3D imaging and High Performance Computing (HPC) to explore and analyse lignocellulosic materials.

The project includes a latest-generation nano-tomograph (EasyTom XL Ultra 150-160 from RX-solutions) achieving a sub-micrometric resolution. This resolution is essential to investigate the cellular morphology at the cell-wall level, the key-scale of plant fibres. Thanks to these 3D digital descriptions, computational models and HPC allow properties and/or behaviours to be predicted on virtual objects. The whole approach is applied to different aspects of biosourced materials: resource characterisation, modelling of elaboration processes, effect of phase morphology on properties, material durability such as mechanical ruin or hydric aging…

After a brief introduction of this project, various observations of plant structures and composites materials will be presented to highlight the potential of the nanotomograph. 3D image processing enables the analysis to go far beyond mere observation. For example, phase segmentation can reveal the morphology of fibres inside a composite.

Then, two examples of simulation on virtual morphologies will be detailed:

- prediction of thermal and water vapour diffusivity of fibreboard panels using high resolution scans and lattice Boltzmann simulations performed with a custom software,
- prediction of permeability in porous structures using OpenFoam, a open-source Computational Fluid Dynamic software.

In the future, we will focus our works on 4D imaging (3D observations during moisture or mechanical loads) for in situ testing of materials, again on real and virtual materials.

The 3D-BioMat project with a total budget of 965,000€ is hosted by the Centre Européen de Biotechnologie et de Bioéconomie (CEBB 51110 Pomacle, France) for a three-year period (from 01/05/2016 to 30/04/2019). This project is co-financed by the Grand Reims (31%) and the European Union by 48.7% (ie 50% of eligible expenditure), committed to the Grand Est with the European Regional Development Fund.

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